U.S. Coast Guard Research and Development Center
1082 Shennecossett Road, Groton, CT 06340-6048

Report No. CG-D-10-03

LARGE-SCALE TESTING OF TREATMENT PROCESSES AS ALTERNATIVES TO BALLAST EXCHANGE

FINAL REPORT
SEPTEMBER 2003

Prepared for:

U.S. Department of Homeland Security
United States Coast Guard
Marine Safety and Environmental Protection (G-M)
Washington, DC 20593-0001

N O T I C E

This document is disseminated under the sponsorship of the Department of Homeland Security in the interest of information exchange. The United States Government assumes no liability for its contents or use thereof.

The United States Government does not endorse products or manufacturers. Trade or manufacturers' names appear herein solely because they are considered essential to the object of this report.

This report does not constitute a standard, specification, or regulation.

Marc B. Mandler, Ph.D.
Technical Director
United States Coast Guard
Research & Development Center
1082 Shennecossett Road
Groton, CT 06340-6048

Technical Report Documentation Page

1. Report No.	2. Government Accession Number	3. Recipient's Catalog No.
CG-D-10-03		

4. Title and Subtitle	5. Report Date / 6. Performing Organization Code
LARGE-SCALE TESTING OF TREATMENT PROCESSES AS ALTERNATIVES TO BALLAST EXCHANGE	September 2003 Project No. 4121.2

7. Author(s)	8. Performing Organization Report No.
Thomas D. Waite, Junko Kazumi, Linda Farmer, Sharon Smith, Peter Lane, Steven Smith, Gary Hitchcock, Thomas Capo, Natalia Zurcher	UDI 456

9. Performing Organization Name and Address		10. Work Unit No. (TRAIS) / 11. Contract or Grant No.
University of Miami Research & Graduate Studies College of Engineering P.O. Box 248294 Coral Gables, FL 33124	U.S. Dept. of Homeland Security United States Coast Guard Research and Development Center 1082 Shennecossett Road Groton, CT 06340-6048	DTCG3900CR0010 DTCG3901CR0007

12. Sponsoring Organization Name and Address	13. Type of Report & Period Covered / 14. Sponsoring Agency Code
Commandant (G-M) U.S. Coast Guard Headquarters Washington, DC 20593-0001	Final Commandant (G-MSO-4) U.S. Coast Guard Headquarters Washington, DC 20593-0001

15. Supplementary Notes

The R&D Center's technical point of contact is LCDR James Hurley, 860-441-2657, email: jhurley@rdc.uscg.mil

16. Abstract (MAXIMUM 200 WORDS)

Large-scale dockside experiments were conducted to evaluate the treatment efficiency of commercially available unit processes for preventing the transfer of unwanted species via ships' ballast water. The project was conducted at the University of Miami (UM) Rosenstiel School of Marine and Atmospheric Science on Virginia Key, Florida. Test system water with natural assemblages of organisms was pumped from Biscayne Bay, Florida, at a flow of approximately 5.7 m^3 min^{-1} (1,500 gpm). Unit processes included a hydrocyclone, a self-cleaning 50 μm screen, and a UV treatment unit. In addition to these unit processes, a mixing and injection system was fabricated to add suspended solids or dissolved coloring agents to the water stream to explore the effect of increased suspended solids (turbidity) or water color on UV treatment efficacy.

The results showed that screening of the test water at 50 μm was effective at removing most of the zooplankton and a small percentage of the microphytoplankton. In contrast, hydrocyclonic separation was not effective for treatment. Initially, UV treatment was able to reduce the count of viable microorganisms to an undetectable level; however, significant regrowth of bacteria was observed. The impact of increased turbidity on all unit processes was considered negligible. Hence, if UV treatment units are to be utilized, removal of suspended solids prior to irradiation may not be necessary if initial design dosage is high enough. At relatively low UV doses, as would be seen in waters of high color, however, the dose was insufficient to inactivate natural assemblages of microorganisms.

17. Key Words	18. Distribution Statement
Ballast Water Treatment, Water Treatment, Aquatic Nuisance Species, Screening, UV Treatment, Hydrocyclone Treatment, Ship Board Treatment	This document is available to the U.S. public through the National Technical Information Service, Springfield, VA 22161

19. Security Class (This Report)	20. Security Class (This Page)	21. No of Pages	22. Price
UNCLASSIFIED	UNCLASSIFIED		

Form DOT F 1700.7 (8/72) Reproduction of form and completed page is authorized.

EXECUTIVE SUMMARY

Large-scale dockside experiments were conducted to evaluate the treatment efficiency of commercially available unit processes for preventing the transfer of unwanted species via ships' ballast water. The project was undertaken utilizing U.S. Coast Guard Research & Development Center funding at the University of Miami (UM) in Coral Gables, Florida. The treatment system was located at UM's Rosenstiel School of Marine and Atmospheric Science on Virginia Key, Biscayne Bay, Miami, Florida. Water with natural assemblages of organisms pumped from Biscayne Bay flowed through the test system at approximately 5.7 m^3 min^{-1} (or 1,500 gpm). Unit processes included a hydrocyclone, a self-cleaning 50 μm screen, and an ultraviolet (UV) treatment unit. In addition to the unit processes, a mixing and injection system was fabricated to add suspended solids or dissolved coloring agents to the water stream to explore the effect of increased suspended solids (turbidity) or water color on UV treatment efficacy.

Treatment efficiency was monitored by evaluating a broad spectrum of biological and biochemical effects. Planktonic organisms, both algae and zooplankton, were monitored to determine any effects due to treatment schemes. In addition, biochemical analyses such as ATP (adenosine triphosphate) were undertaken to determine viability effects of treatment. Also microbial analyses were undertaken to determine effects of UV treatment on the microflora. These analyses were performed to evaluate a wide spectrum of possible effects of treatment on the indigenous organisms.

The results of the analyses showed clearly that hydrocyclonic separation was not effective for treatment at any level in the test water. In addition, it was clear that screening the water stream at 50 μm was effective at removing most of the zooplankton and a small percentage of the micro phytoplankton. The UV treatment was able to reduce the count of viable microorganisms to an undetectable level immediately after treatment. However, regrowth was observed in samples analyzed after 18-hour storage to the level where effectively no net treatment occurred due to UV exposure. In contrast, regrowth was not observed in samples analyzed after longer storage periods (6-day dark storage and 6-day dark storage followed by 24-hour ambient light exposure). It was noted that bacterial abundance in these samples decreased regardless of UV dose indicating that factors other than UV treatment (such as natural causes or grazing by maturing copepods) were responsible for decline in these bacterial numbers over time. There was some indication that the UV treatment affected phytoplankton, although no trends were apparent with respect to destruction of a monitor of phytoplankton biomass (chlorophyll *a*), with either increasing or decreasing UV treatment. This indicates that longer-term grow-out experiments will be required to define these phenomena. Zooplankton viability was not quantified in any of the experiments; however, qualitative microscopic observations of zooplankton groups immediately after UV treatment contained lively specimens regardless of dose, suggesting that UV treatment utilized was not sufficient to rapidly kill mesozooplankton groups present.

Statistical evaluation showed essentially no effect due to increased turbidity throughout the treatment regime, even on the UV treatment unit. The turbidity was varied from approximately 5 NTU (nephelometric turbidity units) to greater than 90 NTU, representing the spectrum that would be encountered in ballasting operations. While the dose delivered by the UV system decreased due to the increased suspended solids loading, its reduced value (approximately

25,000 μW·s cm^{-2} minimum dosage) was sufficient to inactivate microorganisms, even with the increased turbidity. It appears, therefore, that if UV treatment units are to be utilized in ballast water treatment schemes, turbidity removal or suspended solids removal prior to irradiation will probably not be necessary if initial design dosage is high enough. At relatively low UV doses (approximately 10,000 μW·s cm^{-2}) as would be seen in waters of high color (approximately 20 mg L^{-1} humic materials), however, the dose was insufficient to inactivate natural assemblages of microorganisms.

Media filter experiments were also conducted, independent of the large-scale treatment system experiments, using the same water source without altering turbidity or color. These experiments were designed to determine effects of flow rate on particle size distribution (indicative of organism removal) of filtrate. Media of different grain size was used for each of five test runs. It was observed that at increased flow rates, lower percentages of particles were removed, with media type and grain size having little effect. It is evident that for media filters to be utilized in treatment of ballast water, research and optimization of their primary characteristics is needed.

In summary, it was observed that the 50 μm screen contributed appreciably to removal of organisms, especially zooplankton in the test water of the facility. The hydrocyclone was observed to be not effective in removing organisms in the test water. The UV treatment, at doses delivered in these tests, was observed to be not effective at facilitating meaningful treatment of bacteria in test water due to grow-back phenomena observed after 18 hours. Observations indicate that longer-term experiments will be required to define overall effectiveness of UV treatment on phytoplankton and zooplankton.

This research documented performance of selected unit processes in controlled, large-scale tests, and used well-defined analytical methods to obtain statistically based results. Results of these tests provide useful information for development of a defensible scientifically based ballast water treatment standard and also illustrate test methods that should be considered for use in tests and evaluations of ballast water treatment technologies that may be performed in the future.

TABLE OF CONTENTS

SECTION **PAGE**

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF ABBREVIATIONS

AQIS	Australian Quarantine and Inspection Service
ATP	adenosine triphosphate
˚C	degrees Celsius
d	days
˚F	degrees Fahrenheit
g	gravitation constant
gal	gallons
gpm	gallons per minute
gpm ft^{-2}	gallons per minute per square foot
h	hours
IMO	International Maritime Organization
inches Hg	inches of mercury
kPa	kilopascals
L	liters
m	meters
m^3 min^{-1}	cubic meters per minute
mg L^{-1}	milligrams per liter
min	minutes
ml	milliliters
mm	millimeters
MPEC	Marine Environment Protection Committee
mS	milliSiemens
N	normal
N_0	number per volume 0 hours after treatment
N_{18}	number per volume 18 hours after treatment
$N_{Ambient}$	number per volume in untreated ambient water
$N_{Hydrocyclone}$	number per volume after hydrocyclone treatment
nm	nanometers
N_{Screen}	number per volume after screen treatment
NTU	nephelometric turbidity units
Number L^{-1}	number per liter
N_{UV}	number per volume after UV treatment
ppt	parts per thousand
psi in^{-1}	pounds per square inch per inch
s	seconds
T_0	time immediately after treatment
T_{6d}	time six days after treatment
UV	Ultraviolet
δ_0	initial concentration 0 hours after treatment
δ_{18}	concentration 18 hours after treatment
$\delta_{Ambient}$	concentration in untreated ambient water
δ_{UV}	concentration after UV treatment
ε	porosity

LIST OF ABBREVIATIONS (continued)

µg L^{-1}	micrograms per liter
µm	microns
µW·s cm^{-2}	microwatt seconds per square centimeter

Introduction

Ships have plied the earth's waters for centuries, and the use of ballasting materials for ship stability and safety has been common practice since the beginning. As ballast has been transported around the world, so have attached and included organisms. Mills *et al.,* (1993) for instance, have documented over 140 species introduced into the North American Great Lakes since the early 1800s. More than 40 species of this total have been introduced since 1960.

Obviously, by this time, hundreds of nonindigenous species have been introduced around the world via ships' ballast. However, it was the discovery in the 1980s of the European zebra mussel *(Dreissena polymorpha)* in the Great Lakes, a toxic Japanese dinoflagellate *(Gymnodium catenatum)* in Australia, and a North American comb jellyfish *(Mnemniopsis leidyi)* in the Black Sea that brought world wide attention to this problem (Nalepa and Schloesser, 1993, Hallegraeff, 1993, and Ascherson, 1996). These three introductions alone are responsible for damage expenditures of hundreds of millions of dollars.

It was earlier estimated that over 3,000 plant and animal species were being transported daily around the world in ships' ballast water (National Research Council, 1996). However, recent estimates have been upgraded to 10,000 to 15,000 species transported every seven days, with invasion frequencies of one every 24 hours (Carlton, 1999). The potential for severe environmental harm, especially to a nation's natural and farmed resources, is high due to this constant inoculation of nonindigenous species. It has also become clear that catastrophic economic damage can occur if an introduced, invasive specie displaces a region's natural flora or fauna.

Because of the recent awareness of this problem, various national as well as international regulatory agencies have promulgated ballast water management guidelines to help reduce the risk of nonindigenous species introductions. In the United States, for example, the U.S. Coast Guard issued interim rules (effective July 1999) requiring ships operating outside U.S. waters to report their ballast water management practices. This is the most recent measure in the implementation of the *U.S. National Invasive Species Act of 1996.* Also, in February 1999, Executive Order 13112 was issued by the White House requiring all federal agencies to develop procedures for dealing with invasive species, and to form an intergovernmental "Invasive Species Council."

Internationally, the International Maritime Organization (IMO), through its Marine Environment Protection Committee (MEPC), is currently drafting a set of codes and regulations for the control and management of ships' ballast water and sediments to minimize the transfer of harmful aquatic organisms and pathogens. Individual countries, in addition to the U.S., for example, Israel, Australia and Chile, currently mandate ballast water exchange before certain ships can enter their ports. Because Australia has already been heavily impacted by unwanted, invasive species, the Australian Quarantine and Inspection Service (AQIS) has supported a large amount of research on ballast water issues (AQIS, 1994, and AQIS, 1995).

It is clear from the above discussion, that the transport and introduction of nonindigenous species by ships' ballast water poses a significant problem around the world. Currently, there is no effective and coordinated set of procedures to manage this problem, and yet individual countries

are initiating regulations on discharge of ballast water into their ports. In addition, little reliable information currently exists on the true level of *risk* associated with the transport of nonindigenous species between ports. These new and pending port state ballast regulations in the absence of reliable management options could hinder the international free flow of goods via marine shipping.

Other than not ballasting, using clean ballast water, or attempting to predict the *risk* of species introduction via modeling coupled with port monitoring programs, the principal ballast water management options are treatment based. Treatment options can be initially separated into the categories of shore-based or ship-board systems. Shore-based ballast water treatment options have been reviewed by several researchers (Carlton *et al.,* 1995, National Research Council, 1996), and the consensus is that this option is not feasible in the near future. This opinion is based on the lack of infrastructure and space at a typical port, as well as the poor record of creation of shore-based reception facilities for treatment of oily ballast water, an optional method for oil pollution prevention in certain areas under IMO Convention MARPOL 73/78, Annex I.

Ship-board ballast water treatment options can be conveniently classified into three categories: open-ocean ballast water exchange (dilution), separation of suspended material (filtration), and organism inactivation processes (biocide application). A critical review of these options was made by the *Committee on Ships' Ballast Operations,* National Research Council (1996), and to date, the committee's summary remains the most in-depth evaluation of feasible ballast water treatment options. In fact, the *U.S. National Invasive Species Act of 1996,* which amends the *U.S. Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990,* requires that treatment technologies to be explored under the Act follow the recommendations of the National Research Council.

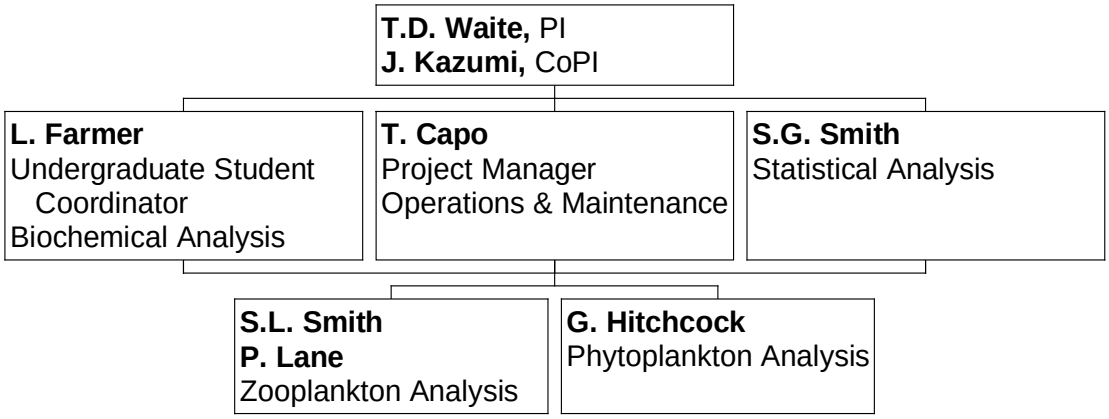
Currently, open ocean ballast exchange (dilution) is the only ship-board management option being utilized to reduce the risk of transfer of unwanted species. The procedure of ballast exchange at sea is practiced in various ways by different ships, resulting in highly disparate levels of dilution. For example, experiments on board the M.V. IRON WHYALLA, utilizing dye dispersion measurements, showed variability in dilution depending on volumes exchanged. A minimum of three (3) tank volumes replaced was required to achieve a 95 per cent exchange, assuming perfectly mixed conditions (AQIS, 1993). Limitations of the effectiveness of ballast exchange as a management tool, including non-ideal mixing, unrecoverable sediments, and short transit times, are well known (Hay and Tanis, 1998). Perhaps the biggest limitation to this management approach however, is the issue of ship safety (National Research Council, 1996, AQIS, 1993), which may in fact preclude this option from being widely adopted.

The ship-board ballast water treatment options, i.e. filtration or biocide application, therefore represent the most promising solutions for lowering the *risk* of introduction of unwanted species via ships' ballast water.

The following describes a demonstration project to evaluate ballast treatment processes receiving attention from developers. The focus was on a self-cleaning strainer, a hydrocyclone, and a biocide. This demonstration was run at a large-scale (5.9 m^3 min^{-1} or 1,500 gpm), and treatment efficiencies were determined by analyses of passing/surviving organisms. These analyses were

undertaken by resident specialists in plankton and microbial ecology at the Rosenstiel School of Marine and Atmospheric Science, University of Miami, Florida.

Management Structure for Project



The ballast water demonstration system was fabricated following a detailed design generated by Dr. Waite. Dr. Waite had created a similar system in Singapore in 1999. The system was constructed by local marine plumbing experts, and the demonstration equipment was shipped to the site and installed by RSMAS personnel as well as technicians from each vendor.

Overview of Treatment Processes

Screening Systems

Pilot scale screening systems have been evaluated for efficiency in treating ballast water, both in fresh water (Cangelosi *et al.,* 1999) and in the marine environment (Matheickal *et al.,* 1999). Both of these demonstrations utilized self-cleaning screens as a technology to prevent the addition of unwanted species into ballast tanks. The efficiency of screens for removing certain classes of organisms, as well as operational constraints of the equipment, were evaluated in these studies. In addition to monitoring materials passing the screens, the rate of fouling of the screens between cleaning cycles was studied for different size openings.

The specific rate of fouling can be determined utilizing pilot plant data for selected mesh sizes and specific water quality, and empirical models have been developed. For example, one model developed by Matheickal *et al.,* (1999) is:

$$(\xi) = k(\theta_0)^{\eta} \qquad (1)$$

where:
ξ = Screening fouling rate, $\delta(Q/A)/\delta t$
θ_0 = Initial hydraulic flux, Q/A
Q = Hydraulic flow through the screen
A = Surface area of the screen
k and η are constants

In linear form: $\ln(\xi) = \ln(k) + \eta \ln(\theta_0)$ (2)

A plot of (ξ) vs. (θ_0) for various initial hydraulic flux values can be developed and the values of k and η can be determined from the slope and y-intercept.

Utilizing the above model, Matheickal *et al.,* (1999) showed that the minimum particle size to be removed becomes a critical parameter in application of this technology. Preliminary data suggest that screen systems can operate efficiently down to particle size cut-offs of approximately 50 μm.

Hydrocyclones

Cyclonic separation has been used for many years in various industrial applications. The process utilizes pre-set flow patterns to separate particles according to mass. Relating these mass separations to average particulate diameters, hydrocyclones have been shown to separate particles of 40 μm to 400 μm in diameter. Basically, a feed stream containing particulate material enters a chamber and rotation is induced such that larger particles migrate downward in a spiral pattern. During this process small particles migrate towards the center and spiral upward and out a separate exit, discharging through an overflow pipe. The higher mass of larger particles remains in the downward spiral path and is discharged as the underflow of the hydrocyclone. Hydrocyclones are currently classified according to the particle size at which 50 per cent ends up in the overflow and 50 per cent in the underflow. This is referred to as the $D50_c$.

Ultraviolet Radiation

Exposure to ultraviolet radiation is a well-recognized method for water treatment and sterilization. UV radiation has disinfection properties that inactivate bacteria, viruses and other microorganisms. In order to inactivate microorganisms, UV rays must strike the cell, pass through the cell body and disrupt its DNA, preventing reproduction. UV treatment does not alter the water chemically nor remove microorganisms from the water. The effectiveness of this process is related to exposure time and lamp intensity as well as general water quality parameters. Exposure time is reported as "microwatt-seconds per square centimeter" ($\mu W \cdot s\ cm^{-2}$).

Methods, Materials and Procedures

Experimental Design, Turbidity Experiments

Experiments were conducted to measure and verify the efficacy of screen or hydrocyclonic filtration and UV treatment for killing or removing marine organisms and microorganisms, including bacteria and other pathogens. Water for the experimental runs was pumped from Bear Cut (Biscayne Bay, Miami, Florida), and the test platform was built on an area adjacent to the

dock. The water in Biscayne Bay typically has a salinity of 32 ppt (range: 28 to 36 ppt), and temperatures of 18 to 28 ^{o}C.

The experimental design included the basic tasks outlined by the U.S. Coast Guard Research & Development Center in each of two contracts. As outlined in Task A, the minimum number of water samples to be collected and analyzed were: 3 sampling points (between the pump and primary treatment; between the primary treatment and secondary UV treatment; after UV treatment) x 3 turbidities (low, medium, high) x 2 time points (0 and 18 h) x 3 replicates = 54 samples. Similarly for Task B, a minimum of 54 samples were collected and analyzed for testing cyclonic filtration followed by secondary UV treatment, for a total of 108 samples (= 2 x 54 samples). Tasks A and B are described below.

Task A: Screen Filtration and Ultraviolet Secondary Treatment

1) The platform, pump, screen filtration, and secondary UV treatment device were configured as shown in Figure 1. The facility included a dockside pump (Marlow Pumps Co., Morton Grove, IL, Model 6E4PEL, self priming), a self cleaning screen (Hayward Industries Co., Elizabeth, NJ, Model 596, 325 mesh, 50 μm stainless steel element), and a UV system (WEDECO-Ideal Horizons Inc., Poultney, VT, Model 1H-60 L). The UV system was comprised of 60 low pressure UV-C germicidal lamps (wavelength 254 nm) arranged in an array of concentric circles within a stainless steel housing. UV dose was monitored by a sensor probe located on the chamber wall at the point of greatest water depth away from the UV lamps. Signal from the sensor was displayed as a relative percentage reading from 0 to 100 per cent on the control panel, and the initial UV output at 100 per cent was determined to be 60,000 $\mu W \cdot s\ cm^{-2}$ by the manufacturer. The UV lamps have an operational life of 9000 h, by which point they have lost approximately 40 per cent of the initial UV output.

2) Triplicate samples were obtained, processed and analyzed as described below:

    a) Samples were obtained prior to filtering, after filtering, before secondary UV treatment and after secondary treatment. As described above, sample ports were located at: i) the inlet pipe to the filter, ii) the outlet pipe from the filter, iii) the inlet pipe to secondary UV treatment, iv) the effluent from secondary UV treatment. Each of the sample ports was flushed for several minutes before the water sample was taken in order to obtain a representative sample. Samples were taken for the various biological and microbiological analyses described below. Each experimental run consisted of running water through the treatment train (filtration followed by UV treatment) at one of the turbidities listed below.

    b) The treatment system was operated at a flow of approximately 1500 US gpm, and a filter size of 50 μm.

    c) The treatment system was operated at 3 turbidities: i) low - ambient Biscayne Bay water, ii) medium - ambient Biscayne Bay water with slightly raised turbidity, and

iii) high - ambient Biscayne Bay water with greatly raised turbidity. The turbidity was raised by adding a model clay, kaolinite, via injection pump located prior to the turbidity meter and the inlet to the filter unit. A kaolinite/water slurry was mixed in a 130 L (35 gal) polypropylene tank and injected into a PVC in-line mixer (Cole Parmer Company, Inc., Vernon Hills, IL) as required. The turbidity was monitored throughout the experiment to ensure constant turbidity.

d) Samples were analyzed for the various biological and microbiological indices, as described below, immediately and 18 h after the experimental run. Two sets of samples were collected during each experimental run: one set was analyzed immediately, and the second set was kept for 18 h at ambient temperature prior to analysis in order to determine the long term effects of treatment.

3) Samples were analyzed to determine the presence or absence of various species, with an estimate of viability. The analyses included the amount of organisms removed or killed at each stage of treatment. Please see Biological Protocols section for further information.

4) The data were analyzed to determine the efficacy of the filter and secondary UV treatment, and the effect of turbidity.

Task B: Cyclonic Filtration and Ultraviolet Secondary Treatment

1) Platform, pump, piping with sampling ports, and catchment reservoirs were configured as shown in Figure 1.

2) A hydrocyclonic filtration apparatus (Krebs Engineers, Tucson, AZ, Model KSH-20-1437), and secondary UV module were used. The hydrocyclone had a centrifugal force of approximately 13 X g at 5.7 m^3 min^{-1} (1500 gpm). The UV unit was the same as in Task A. (See Figure 1).

3) Triplicate samples were obtained, processed and analyzed as described in Task A.

4) Samples were analyzed to determine the presence or absence of various species, with an estimate of viability. The analysis included the amount of organisms removed or killed at each stage of treatment.

5) The data were analyzed to determine the efficacy of the hydrocyclonic filter and secondary UV treatment, and the effect of turbidity.

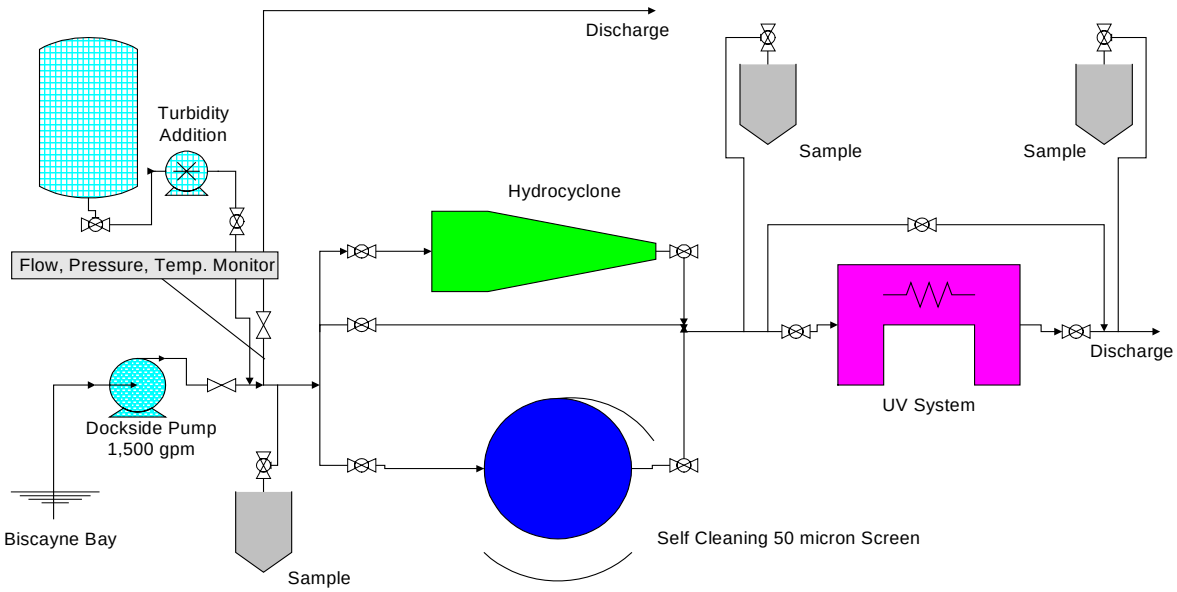


Figure 1. Schematic of ballast water treatment system used for turbidity experiments.

Experimental Design, Water Color Experiments

These experiments were designed to study the effects of color on the efficiency of the screen filtration/UV treatment processes. The minimum number of water samples collected and analyzed at one test condition (or color level) was: 3 sampling points (between the pump and screen filter; between the screen filter and UV treatment chamber; after the UV treatment chamber) x 3 time points (0 h, 6 d, and 6 d + 24 h) x 5 replicates = 45 samples. Similarly, a minimum of 45 samples each was collected and analyzed for the second and third test conditions of reduced water transmission, for a total of 135 samples (= 3 x 45 samples).

Tests were conducted at ambient turbidity (2-5 NTU), using three conditions of water color (ambient and two conditions of reduced water transmission). Samples were analyzed immediately after treatment, after 6 d of dark incubation under controlled temperature, and again after 24 h exposure to natural light. The water color experiments are described below.

Task C: Screen Filtration and Ultraviolet Secondary Treatment

1) The test platform with a pump, filtration unit and a UV treatment process was the same as used in the previous tasks. Three more tanks and the necessary piping and sampling ports were installed in order to accommodate the sampling requirements (See Figure 2).

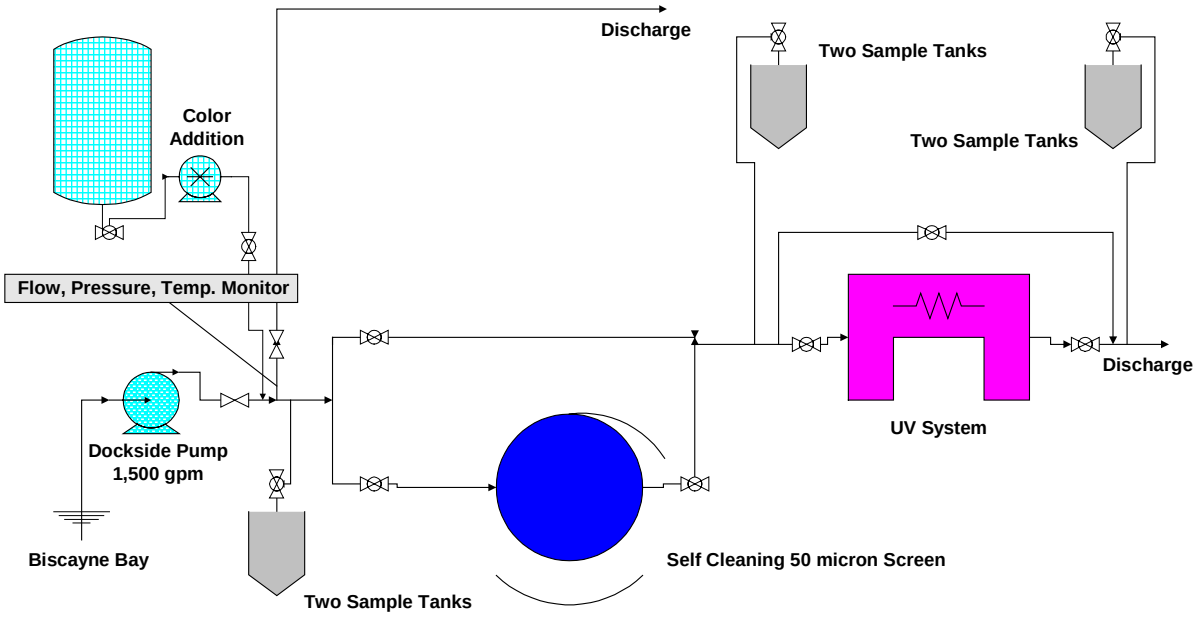


Figure 2. Schematic of ballast water treatment system modified for water color experiments.

2) Experiments with three separate conditions of water color (ambient and two conditions of reduced water transmission) were conducted. For each of the three test conditions, five independent runs were conducted, as described below.

a. Test Condition I (5 runs)
   Pump flow rate: approximately 1500 gpm (U.S.)
   Filter screen size: 50 µm
   Turbidity: ambient (8 NTU maximum)
   Water color: ambient
   UV dosage: 60,000 µW s cm^{-2}

b. Test Condition II (5 runs)
   Pump flow rate: approximately 1500 gpm (U.S.)
   Filter screen size: 50 µm
   Turbidity: ambient (8 NTU maximum)
   Water color: coloring agent added to produce 75 per cent UV transmission
   UV dosage: 45,000 µW s cm^{-2}

c. Test Condition III (5 runs)
   Pump flow rate: approximately 1500 gpm (U.S.)
   Filter screen size: 50 µm
   Turbidity: ambient (8 NTU maximum)
   Water color: coloring agent added to produce 17 per cent UV transmission
   UV dosage: 10,000 µW s cm^{-2}

3) Preliminary experiments with coloring agents, including instant coffee, yellow food coloring and commercially available humic materials showed that at the concentrations required to reduce UV transmission to the desired levels, these substances were toxic to marine organisms. Thus, humic materials (humic acids, sodium salt, H16752, Sigma Aldrich Co, St. Louis MO) were added in sufficient quantities to achieve the desired UV doses at the test facility, and a standard curve of UV dose vs. humic materials concentration was generated (Figure 3). Collected water samples were also analyzed for color by the Visual Comparison Method (Method 2120 B; *Standard Methods for the Examination of Water and Wastewater,* American Public Health Association, 1998). To achieve the targeted UV doses, lamps in the UV unit were disconnected, and the per cent UV transmittance continuously monitored during the test runs to assure constant conditions.

4) Representative samples were collected in 200 gal holding tanks from each sample port for each of the 15 test runs (= 3 test conditions x 5 replicate test runs). Each of the sample ports was flushed for several minutes before the water sample was taken in order to obtain a representative sample.

5) For each test run, samples representative of water immediately after treatment, after a period of darkness and after release into the environment were collected. Immediately after collection of the large volume samples in the three holding tanks, water samples were taken for the various biological and biochemical analyses as described below. These were the "T_0" samples. The water (approximately 200 gal) in the holding tanks were then emptied through 35 μm Nitex mesh plankton nets to concentrate the samples for zooplankton analysis.

    a. After the "T_0" samples were taken, six tanks were filled simultaneously in order to accommodate the sampling requirements for the biological protocols. All the tanks were covered with opaque plastic sheeting and incubated in the dark at ambient temperatures to simulate residence in a ballast water tank. The ambient water temperature during the test period (February - June) was continually monitored by temperature sensors suspended in the tanks. The temperature averaged approximately 25 ^{o}C (or 77 ^{o}F) during the test period.

    b. After the dark environment incubation for 6 d, samples were taken from three of the holding tanks and analyzed for the various indices, as described below. These were the "T_{6d}" samples. The water (approximately 200 gal) in the three holding tanks were then emptied through 35 μm Nitex mesh plankton nets to concentrate the samples for zooplankton analysis.

    c. The covers from the remaining three holding tanks were removed such that the tanks were exposed to outdoor natural light for a period of 24 h.

    d. After natural light exposure for 24 h, samples were taken from the remaining three holding tanks and analyzed for the various indices, as described below. These were the "T_{6d+24h}" samples. The water (approximately 200 gal) in the three

holding tanks were then emptied through 35 µm Nitex mesh plankton nets to concentrate the samples for zooplankton analysis.

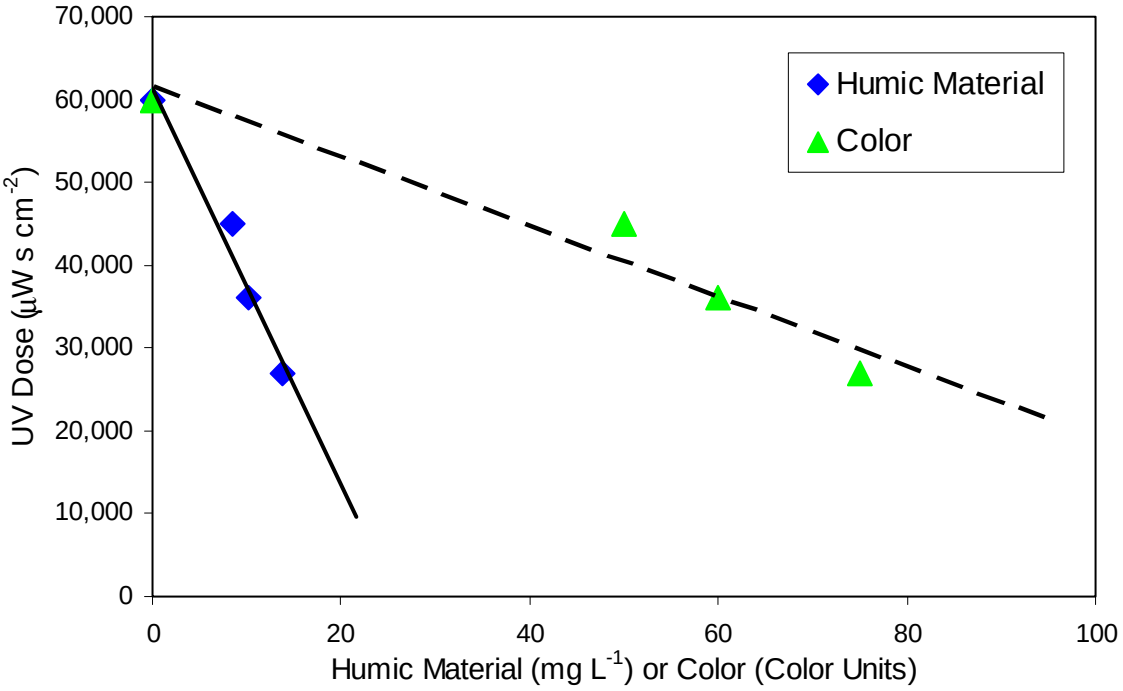


Figure 3. Effect of humic material concentration or color on UV dose.

Biological Protocols and Statistical Analysis

Representative water samples were collected in 200 gal holding tanks from each sample port for each of the test runs. Each sample port was flushed for several minutes before the water sample was taken in order to obtain a representative sample. Immediately after collection of the large volume samples, two water samples (approximately 1 L each) were taken from each tank for the various analyses as described below. The remaining water (approximately 200 gal) in the holding tanks was then emptied through 35 µm Nitex mesh plankton nets to concentrate the samples for zooplankton analyses. Organisms that were retained by the 35 µm net were zooplankton and other macrobiota, while those that passed through the net (< 35 µm in size) were chiefly bacteria and phytoplankton.

Biochemical Analysis for Viability of Organisms

ATP content was used to assess the viability of organisms that were not removed by filtration. ATP is produced by all living organisms and is rapidly degraded by ATPases with cell death. This analysis has wide application in the determination of living biomass in sediments (Karl and LaRock, 1975), sludge (Patterson *et al.,* 1970), marine water columns (Holm-Hansen and Booth, 1966, Maranda and Lacroix, 1983), as well as in phytoplankton (Holm-Hansen 1969, Hitchcock *et al.,* 1987) and bacterial populations (Lundin and Thore, 1975).

The size fractionated samples (both >35 μm and <35 μm) were immediately placed in boiling Tris buffer for extraction to avoid ATPase activity (Cheer *et al.,* 1974). Once ATP is released, samples may be frozen with little loss of activity (Patterson *et al.,* 1970). ATP was analyzed with the Luciferin-luciferase assay (Holm Hansen and Booth, 1966) using a Turner TD20/20 luminometer. In order to relate ATP values to biomass, protein content was also measured on the same samples analyzed for ATP using a heated biuret-Folin assay (Dorsey *et al.,* 1978).

Microbiological Analysis

Microbiological testing of water for drinking and recreational uses is continually conducted by various local, state and federal agencies. Methods for enumeration and viability of indicator microorganisms are fairly well established, and most agencies follow the protocols stated in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1998).

Water samples were analyzed for total cultivable heterotrophic bacterial counts (Method 9215 D, Heterotrophic Plate Count, Membrane Filter Method), total coliforms and *Escherichia coli* (Method 9223 B, Enzyme Substrate Coliform Test). For total cultivable heterotrophic bacterial counts, 1 or 10 ml of seawater sample were filtered through 0.45 μm filters, and the filters placed on NWRI agar plates. The plates were incubated for 5 d at 24 °C before counting visible colonies. Total coliforms and *E. coli* were enumerated using Colilert 18® (IDEXX Laboratories, Inc., Westbrook ME), a commercially available kit approved by the Standard Methods Committee for use in Method 9223. We used standard microbiological techniques as outlined in Method 9030 (Laboratory Apparatus), 9040 (Washing and Sterilization) and 9050 (Preparation of Culture Media).

Phytoplankton Analysis

Phytoplankton pigment analyses provide an index to the biomass of viable phytoplankton through estimates of chlorophyll *a* in living cells. An index to the detrital, or dead, material was provided by the fluorometeric measurement of phaeophytin, which is actually composed of a suite of chlorophyll degradation products (Smith *et al.,* 1981). Our method for the analysis of chlorophyll *a* and phaeophytin was based on Method 445.0 of the U.S. Environmental Protection Agency (Arar and Collins, 1992). The stated detection limit was 0.05 μg L^{-1} for chlorophyll *a* and 0.06 μg L^{-1} for phaeophytin in marine waters. Particulate matter was collected by filtration of three replicates (sample volume of 100 ml) at a vacuum of 5 inches Hg (< 20 kPa) onto Whatman GF/F filters. If the analyses were not conducted immediately, the filters were stored in individual plastic vials in a desiccator at –20 °C. Within three weeks the filters were ground in a tissue grinder in 5 ml of 90 per cent acetone, and the slurry was then transferred to a 15 ml polyethylene test tube. A blank filter was included in the extraction process and analyzed to detect potential contamination of reagents or possible problems with the instrumentation. The capped test tubes were placed in the dark at 4 °C for 24 h to extract the pigments. This extraction period was sufficient to provide a Relative Standard Deviation of 5.0 per cent on replicate samples (Table 1, USEPA Method 445.0).

The slurry was centrifuged at 1000 g for 5 min to clear the supernatant following the extraction period. The acetone was transferred to a 4 ml glass cuvette and fluorescence was measured before and after acidification (0.1 ml of 0.1 N HCl) on a Turner Designs Model 10 fluorometer. The instrument was equipped with a F4T5 blue lamp, a red-sensitive photomultiplier, a Corning CS-5-60 excitation filter, and a Corning CS-2-64 emission filter in accord with the USEPA Standard Method 445.0. The initial reading reflected the combined contribution of 'total' chlorophyll *a* while the final (acidified) reading was primarily phaeophytin (Smith *et al*., 1981). The instrument was calibrated with a Standard Solution made from pure chlorophyll *a* obtained from Sigma Chemical Corp (cf. Section 10.0, Calibration and Standardization, USEPA Method 445.0). The purity and concentration of the Standard Solution was checked by spectrophotometric methods during each bimonthly calibration. Concentrations are reported in triplicate as $\mu g\ L^{-1}$ for both chlorophyll *a* and phaeophytin.

Zooplankton Analysis

Zooplankton samples were collected from 35 μm mesh nets suspended in each of the three sample collecting tanks (pre-treatment, post-screen or hydrocyclone treatment, and post-secondary UV treatment). The nets were custom manufactured by Sea-Gear Corporation with a net mouth diameter of 0.75 m and a length of 1.45 m to allow suspension in the 200 gal collecting tanks. The net cod-ends, which contained approximately 300 ml of sample, were rinsed into 1 L beakers and made up to 500 ml with filtered sea water for subsequent laboratory processing.

In the laboratory 150 ml was taken from each beaker for ATP analysis (see section on Biochemical Analysis), with the remaining 350 ml rinsed into a 500 ml glass jar, treated with Neutral Red dye (JT Baker) for several minutes, and finally preserved with a 4 per cent buffered (sodium borate) formaldehyde solution. All samples were allowed to stand for at least two days prior to taxonomic enumeration to allow absorption of the stain. In addition, nearly all zooplankton samples were sub-sampled prior to staining and fixation for brief microscopic surveys of apparent vitality of organisms present.

Laboratory analyses of samples for taxonomic enumeration involved splitting each zooplankton sample several times in a Folsom splitter to obtain aliquots containing approximately 200-400 individuals. Three aliquots were counted with the aid of a Leica Wild M10 or Leica MS5 stereomicroscope for numerically dominant mesozooplankton taxa and groups. When an aliquot contained more than approximately 50 specimens of a species or taxon, that taxon was not counted in subsequent aliquots. The composition and number of species present determined the size of the second and third aliquots. For example, if the first aliquot contained 400 organisms of which 300 were small, unidentified copepod nauplii, then a larger aliquot was utilized for subsequent counts in order to obtain greater numbers of other species or groups. This method has been used in numerous studies conducted previously by Smith and Lane (e.g., Smith *et al.,*1985, Smith and Lane, 1988, Flagg and Smith, 1989, Lane *et al.,* 1993, 1995, 1996, Ashjian *et al.,*1995, 1997) and conforms in general with other recently published zooplankton sample enumeration guidelines (Postel *et al.,* 2000). Previous net samples collected from the dock at the Rosenstiel School have often been dominated numerically by various stages of the small calanoid copepods *Acartia tonsa* and *Paracalanus* spp., and the small cyclopoid copepod genus

Oithona (Lane, unpublished data). We identified these and any other numerous copepods to the genus level. Other mesozooplanktic groups including chaetognaths; appendicularia; mysiids; and larvae of the decapod, echinoderm and polychaete groups were counted if they were observed in samples.

Experimental Design and Statistical Analysis

A standard paired-sample design was employed in which samples taken pre- and post-primary unit process, and pre- and post- UV treatment during a single experimental run were considered as respective sample pairs. This design is a special case of a Randomized Complete Block design where each experimental run is considered as a separate block (Montgomery 1997). Experimental treatments were the primary unit process type (50 μm screen or hydrocyclone) and corresponding UV treatment. The paired-sample block design was chosen to control for variation in response variables (e.g., ATP, total coliforms, chlorophyll *a*, etc.) among experimental runs, since each run was conducted at a different date and time and the seawater for each run was drawn from the natural environment. For the turbidity experiment, turbidity level (low, medium, high) and sample analysis time (0 h and 18 h after sampling) were incorporated as additional blocking variables in a factorial arrangement. For the water color experiment, water color (low, medium, high) and sample analysis time (0 d, 6 d and 6 d + 24 h after sampling) were incorporated as additional blocking variables in a factorial arrangement.

For the turbidity experiment, statistical analyses for each response variable assessed two main aspects of unit process performance at the 0 h time point: i) the performance of 50 μm screen and hydrocyclone primary unit processes; and ii) the additional performance of UV treatment for each primary unit process. To understand the influence of sample analysis time, statistical analyses focused on assessing the change in a given response variable 18 h after three different levels of treatment, namely i) no treatment, ii) primary treatment, and iii) primary treatment + UV treatment, for each treatment system (50 μm screen or hydrocyclone). The following additional aspects of performance were evaluated for both 0 h and 18 h time points using a factorial analysis: i) comparison of performance between treatment systems (50 μm screen vs. hydrocyclone); and ii) turbidity influence on treatment system performance.

For the water color experiment, statistical analyses for each response variable assessed the performance of 50 μm screen and the additional performance of UV treatment at T_0. Factorial analysis was used to evaluate each of these aspects with respect to the influence of UV transmission level and sample analysis time. To understand the influence of sample analysis time, statistical analyses focused on assessing the change in a given response variable 6 d and 6 d + 24 h after three different levels of treatment, namely i) no treatment, ii) primary treatment (50 μm screen), and iii) primary treatment + UV treatment.

For both turbidity and water color studies, statistical inference and hypothesis testing was conducted using the General Linear Model analysis framework (e.g., ANOVA, linear regression) for normally-distributed or transformed-normal response variables (Neter et al. 1996). The normality assumption was evaluated by: i) inspection of frequency histograms of general linear model error residuals, and ii) application of the Shapiro-Wilk test (Shapiro & Wilk 1965). When necessary, response variables were corrected for normality using either the natural logarithm or

square root transformation. All statistical analyses and modeling were performed using the Statistical Analysis System software package (SAS Institute, Cary, NC).

Media Filter Experiments

In these experiments, seawater was passed through various kinds of media to determine differences among particle size distributions. In addition, variations in size distribution of particles due to different flow rates were analyzed. A Beckman Coulter Multisizer 3® (Beckman Coulter Inc., Miami, FL) with a 400 μm aperture (particle size range: 8 to 240 μm) was used for all particle analyses. Prior to sample analyses, calibration of the instrument was conducted as per the manufacturer's instructions.

Sampling Procedure

A media filter was installed at the Ballast Water Treatment Test Facility. A specific media grain was tested each sampling day. An average of five samples at different loading rates were taken during each sample day, including an ambient water sample. After transporting back to the lab, the samples were stored in the refrigerator to minimize degradation.

The filtration depended on the media grain size diameter. The porosity (ε) obtained determined the filtration efficiency. Five different kinds of media were used: Coarse Sand (0.80 to 1.2 mm), Fine Sand (0.45 to 0.55 mm), Anthracite (0.95 to 1.05 mm), Silica Sand (0.90 to 1.0 mm) and Silica Sand (0.30 to 0.45 mm). The porosity (ε) was calculated by the following equation:

$$\varepsilon = \text{Void Volume / Bed Volume}$$
$$= 500\ \text{ml} - [\text{Weight of Media (g)} / \text{Specific gravity of Media (g ml}^{-1})] / 500\ \text{ml},$$

where 500 ml is the fixed volume used.

Table 1 shows the calculations for the specific porosities for each Media type.

Table 1. Porosity calculations for each Media type.

Media type	Calculated porosity
Silica Sand (0.30 mm to 0.45 mm)	ε = [500 – (783.36 / 2.65)] / 500 = 0.40
Silica Sand (0.45 mm to 0.55 mm)	ε = [500 – (755.00 / 2.65)] / 500 = 0.43
Silica Sand (0.8 mm to 1.2 mm)	ε = [500 – (847.10 / 2.65)] / 500 = 0.36
Silica Sand (0.9 mm to 1.0 mm)	ε = [500 – (814.33 / 2.65)] / 500 = 0.38
Anthracite (0.95 mm to 1.05 mm)	ε = [500 – (461.54 / 1.50)] / 500 = 0.38

Analytical Procedure

Before starting sample analysis using the particle size counter, an electrolyte or Standard Saline Solution was made and analyzed to characterize the background. This background run was an analysis of the electrolyte without sample material and was also referred to as a "Blank Analysis". When analyzing low concentration samples, a background run was essential. The electrolyte solution had a conductivity of 50.0 mS (milliSiemens) and a salinity of 36 ppt (for exact NaCl values and preparation, refer to Appendix E), and provided the required matrix for running the sample. To prevent contamination of this solution and to decrease the amount of background noise in each run, the saline solution was filtered manually through a 0.22 μm Millipore® filter. A background was run for every batch of samples to be analyzed in a single day using the same electrolyte. After filtering the electrolyte and running the background, the samples were analyzed.

Results and Discussion

The large-scale dockside ballast water treatment system was tested over the span of approximately one year on natural sub-tropical waters. The system included a hydrocyclone, self-cleaning screen (50 μm) and a UV treatment unit. The basic test protocol was to evaluate changes in natural seawater after each unit process in the treatment system. While the general quality of the seawater varied somewhat (e.g. turbidity range: 1.3 to 5.3 NTU) over the period of testing, the treatment efficiency of each process was statistically determined utilizing a difference technique, thereby normalizing the variability associated with ambient seawater conditions. In order to test the treatment capability of the unit processes under demanding conditions, the suspended solids content of the ambient seawater was augmented by the addition of clay materials. The intent was to mimic severe turbid conditions that might be encountered by ballast water treatment equipment. The suspended solids content was monitored via turbidity measurements (NTU), and the test conditions ranged from 2 to 80 NTU. It was expected that the increased suspended solids content, and therefore, reduced clarity of the seawater, would most affect the UV treatment process. Similarly, a second set of experiments using the screen and UV unit were conducted with reduced UV doses that would be expected when treating natural waters of high water color.

The hydrocyclone and self-cleaning screen systems are physical removal treatment processes, while the UV treatment unit induces biocidal effects. Because of these distinctions, there was a slight modification in the test protocol to reflect expected treatment effects. For example, since zooplankton viability was not quantified in any of the experiments, evaluation of these populations after UV treatment was not required. UV treatment was also not expected to have detrimental effects to larger organisms such as zooplankton, and visual observations of samples taken after UV treatment noted actively swimming zooplankton. Additionally, zooplankton populations were not enumerated for the 18 h regrowth experiments because the time frame is considered too short for substantial growth and reproduction of zooplankton to be detectable, thus no changes were expected. In the second experiment with the 6 d dark storage time, zooplankton were enumerated, but again, not in the samples obtained after exposure to 24 h of light after the 6 d dark period.

The following discussion presents the main findings of the study. Complete details of the experimental data and all statistical analyses are provided in Appendices A to D. Statistical evaluation of treatment efficiency for the screen, hydrocyclone and UV unit processes tested are presented in Table 2. This table shows a comparison of these unit processes, as well as any impact on each unit process due to turbidity effects. For the paired-sample experimental design the main variable used in statistical tests was *d*, the difference in response variable amount before and after treatment by a given unit process. Statistical significance for a unit process indicated that mean *d* was different from zero. Statistical significance for turbidity impact indicated that mean *d* differed among turbidity levels (low, medium, high). Again, note that enumeration of zooplankton was omitted in those test conditions where no discernable effect was anticipated.

Table 2 also shows that the physical separation processes, i.e., the 50 μm screen and the hydrocyclone, behaved differently with respect to reductions of those organisms that are affected by these systems, namely the zooplankton groups (*Acartia*, *Paracalanus*, *Oithona*, Harpacticoida, Copepoda nauplii, gastropod and bivalve larvae). Statistically significant reduction of all zooplankton groups monitored occurred with the screen ($p < 0.01$), while very little reduction occurred with the hydrocyclone. There was some reduction in both gastropod and grouped invertebrate larvae with the hydrocyclone ($p < 0.01$), otherwise, little removal was observed.

In all cases, the UV treatment unit was capable of reducing the populations of bacteria (total cultivable heterotrophic bacteria, total coliforms, *Escherichia coli*) significantly ($p < 0.01$), and in general, no turbidity effects were noted. The observation that no significant effects were seen with increased turbidity, hence decreased UV treatment, is due to the fact that even at elevated turbidity loadings, sufficient light was still available to inactivate bacteria. For example, at the highest suspended solids concentrations (80 NTU), there was still in excess of 25,000 $\mu W \cdot s\ cm^{-2}$ UV dose measured. This dose has been reported in other studies to reduce bacterial numbers significantly, and indeed reduction in bacterial density was also noted during our tests. Because the UV treatment system utilized in our experiments was designed to deliver a dose in excess of 60,000 $\mu W \cdot s\ cm^{-2}$ in ambient seawater, even at the highest concentration of solids used, the radiation dose could not be reduced to a level such that effects on bacterial numbers could be observed. It also should be noted here, that the test range of added suspended solids (up to 80 NTU) is in excess of any found in the natural environment. Generally speaking, the most turbid waters naturally encountered are in the range of 10 to 15 NTU.

Table 3 shows the statistical evaluation for the test system comprising the 50 μm screen plus UV, and considering the 18 h incubation period allowed after treatment. Once again, there is virtually no effect from turbidity enhancement on each of the unit processes. Over the entire test scenario, the only significant effects were noted for UV treatment on bacteria, and to some extent, on chlorophyll *a* or phytoplankton biomass. Significant changes were observed in both bacterial and phytoplankton biomass and activity after the 18 h incubation ($p < 0.05$). In particular, a significant increase in bacterial numbers occurred while significant decreases in phytoplankton biomass occurred during the 18 h incubation.

Table 4 shows the statistical evaluation of data collected after an 18 h incubation period for the hydrocyclone plus UV treatment system. Once again, virtually no significant changes in any of

Table 2. Response variables vs. efficiency of screen, hydrocyclone and UV unit processes (Time = 0 h).

	Unit process							
	Screen		Screen + UV		Hydrocyclone		Hydrocyclone + UV	
Response variable	Turbidity impact	Significant reduction	Turbidity impact	Significant reduction	Turbidity impact	Significant reduction	Turbidity impact	Significant reduction
Total Bacteria	N	N	N	**	N	*	N	***
Total Coliforms	N	N	N	***	N	N	*	***
E. coli	N	N	N	***	N	N	N	***
Total Chlorophyll *a*	N	N	N	N	N	N	*	**
Total Phaeophytin	N	N	N	N	N	N	N	N
Acartia spp. (Order Calanoida)	N	***	ND	ND	N	N	ND	ND
Paracalanus spp. (Order Calanoida)	N	**	ND	ND	*	N	ND	ND
Oithona spp. (Order Cyclopoida)	N	***	ND	ND	N	N	ND	ND
Order Harpacticoida	N	***	ND	ND	N	N	ND	ND
Class Copepoda Nauplii	N	**	ND	ND	N	N	ND	ND
Class Gastropoda Larvae	N	***	ND	ND	N	**	ND	ND
Class Bivalvia Larvae	N	**	ND	ND	N	N	ND	ND
Grouped Invertebrate Larvae	N	***	ND	ND	N	**	ND	ND
ATP (> 35 µm)	-	-	-	-	-	-	-	-
ATP (< 35 µm)	-	-	-	-	-	-	-	-
Protein (> 35 µm)	N	***	-	-	N	*	-	-
Protein (< 35 µm)	-	-	-	-	-	-	-	-

*: significant at < 0.05
**: significant at < 0.01
***: significant at < 0.001
N: no effect
ND: not determined
-: insufficient data for statistical analysis
Total Bacteria: total cultivable heterotrophic bacteria
Grouped Invertebrate Larvae: includes gastropod, bivalve, decapod and echinoderm larvae

Table 3. Response variables vs. efficiency of screen with UV system (Time = 18 h).

Response variable	Unit process: Ambient water – Turbidity impact	Ambient water – Change after 18 h	Screen – Turbidity impact	Screen – Change after 18 h	Screen + UV – Turbidity impact	Screen + UV – Change after 18 h
Total Bacteria	N	*, I	*	-	N	*, I
Total Coliforms	N	N	N	N	N	**, I
E. coli	N	N	N	N	N	*, I
Total Chlorophyll *a*	N	*, I	N	*, I	N	**, D
Total Phaeophytin	N	N	N	**, D	N	***, D
Acartia spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
Paracalanus spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
Oithona spp. (Order Cyclopoida)	ND	ND	ND	ND	ND	ND
Order Harpacticoida	ND	ND	ND	ND	ND	ND
Class Copepoda Nauplii	ND	ND	ND	ND	ND	ND
Class Gastropoda Larvae	ND	ND	ND	ND	ND	ND
Class Bivalvia Larvae	ND	ND	ND	ND	ND	ND
Grouped Invertebrate Larvae	ND	ND	ND	ND	ND	ND
ATP (> 35 µm)	-	-	-	-	-	-
ATP (< 35 µm)	-	-	-	-	-	-
Protein (> 35 µm)	N	N	N	N	N	N
Protein (< 35 µm)	N	N	N	*, D	N	N

*: significant at < 0.05
**: significant at < 0.01
***: significant at < 0.001
I: increase
D: decrease
N: no effect
ND: not determined
-: insufficient data for statistical analysis
Total Bacteria: total cultivable heterotrophic bacteria
Grouped Invertebrate Larvae: includes gastropod, bivalve, decapod and echinoderm larvae

Table 4. Response variables vs. efficiency of hydrocyclone and UV system (Time = 18 h).

Response variable	Unit process					
	Ambient water		Hydrocyclone		Hydrocyclone + UV	
	Turbidity impact	Change after 18 h	Turbidity impact	Change after 18 h	Turbidity impact	Change after 18 h
l Bacteria	N	N	N	N	N	***, I
l Coliforms	N	N	N	N	N	***, I
oli	N	N	N	N	N	*, I
l Chlorophyll *a*	N	N	N	*, I	*, D	***, D
l Phaeophytin	N	*, D	N	*, D	N	***, D
rtia spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
acalanus spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
ona spp. (Order Cyclopoida)	ND	ND	ND	ND	ND	ND
er Harpacticoida	ND	ND	ND	ND	ND	ND
s Copepoda Nauplii	ND	ND	ND	ND	ND	ND
s Gastropoda Larvae	ND	ND	ND	ND	ND	ND
s Bivalvia Larvae	ND	ND	ND	ND	ND	ND
uped Invertebrate Larvae	ND	ND	ND	ND	ND	ND
(> 35 µm)	-	-	-	-	-	-
(< 35 µm)	-	-	-	-	-	-
ein (> 35 µm)	N	N	N	N	N	N
ein (< 35 µm)	N	N	N	N	N	N

ficant at < 0.05
ificant at < 0.01
gnificant at < 0.001
ase
ease
ffect
t determined
ficient data for statistical analysis
acteria: total cultivable heterotrophic bacteria
d Invertebrate Larvae: includes gastropod, bivalve, decapod and echinoderm larvae

the measured parameters were observed other than after UV treatment. Similar to the results reported above for screen plus UV, it is noted that a significant increase in growth of bacteria, and significant decrease in phytoplankton biomass occurred after the 18 h incubation after treatment with hydrocyclone and UV.

Figure 4 is a graphic representation of the treatment effects on bacterial populations monitored in ambient seawater. Because there were no turbidity effects (see Tables 2 and 3), all the data were grouped together for this analysis. It can be seen that the relative concentrations of total cultivable heterotrophic bacteria and coliforms remain unchanged from the ambient seawater through the physical separation processes. This result was expected, as neither of these processes was intended to remove material the size of bacteria (1 to 2 μm). It is also noted that the UV system facilitated significant removal of bacteria, with essentially all of the coliforms (total and *E. coli*) being removed to below detection levels (10 organisms per 100 ml), and only a small residual of total cultivable heterotrophic bacteria remaining in the water. These data indicate that UV will be effective at significantly reducing bacterial populations, and that the effects of pretreatment via screens or hydrocyclones probably will not be required to enhance removal efficiencies of a UV system. It appears that a UV system could be designed to effectively handle typical ballast water, regardless of the suspended material in the water, if bacteria are the only or primary target organisms to be removed.

It was initially expected that elevated turbidities would affect UV efficiency. Figure 5 and Table 5 show the effect of added turbidity in the test system on bacterial inactivation from UV treatment. It can be seen that the radiation dose delivered by the UV system varied from approximately 60,000 $\mu W \cdot s\ cm^{-2}$ at ambient turbidity to 25,000 $\mu W \cdot s\ cm^{-2}$ at 80 NTU elevated turbidity from added suspended solids. There is little effect the different radiation doses have on inactivation of any of the bacterial groups tested. As discussed previously, this indicates that a dose of even 25,000 $\mu W \cdot s\ cm^{-2}$ is sufficient to significantly reduce bacterial populations in natural water systems.

It is well known that genetic damage induced by UV treatment can be repaired, and this can be expressed as microorganism regrowth with time. This repair phenomena was observed in a series of tests undertaken during the project period. Figure 6 and Table 6 show the results of these regrowth experiments. It can be seen that bacterial populations, here depicted as the relative population present after 18 h (N_{18} / N_0), exhibited significant regrowth after UV treatment, regardless of the dose delivered. In fact, it was noted that the viable population of all bacterial groups tested after 18 h of regrowth was between 10 and 100 times the population monitored directly after the UV treatment event at 0 h. Obviously, this is a significant issue that must be addressed if UV treatment is to be considered as a treatment technology to reduce the population of microorganisms in ballast water. In addition, it should be noted that typical water treatment technologies require that treatment efficacy be substantial and far in excess of that required to reduce natural populations of microorganisms by 2 to 3 orders of magnitude. For example, if water is to be treated to remove bacterial populations that are present in the range of 10^3 organisms per ml, then treatment to facilitate at least a six log reduction is required in order for that treatment process to be reliable and viable. Therefore, if UV is to be utilized as a significant treatment process to reduce bacterial populations, the dose will need to be in excess of

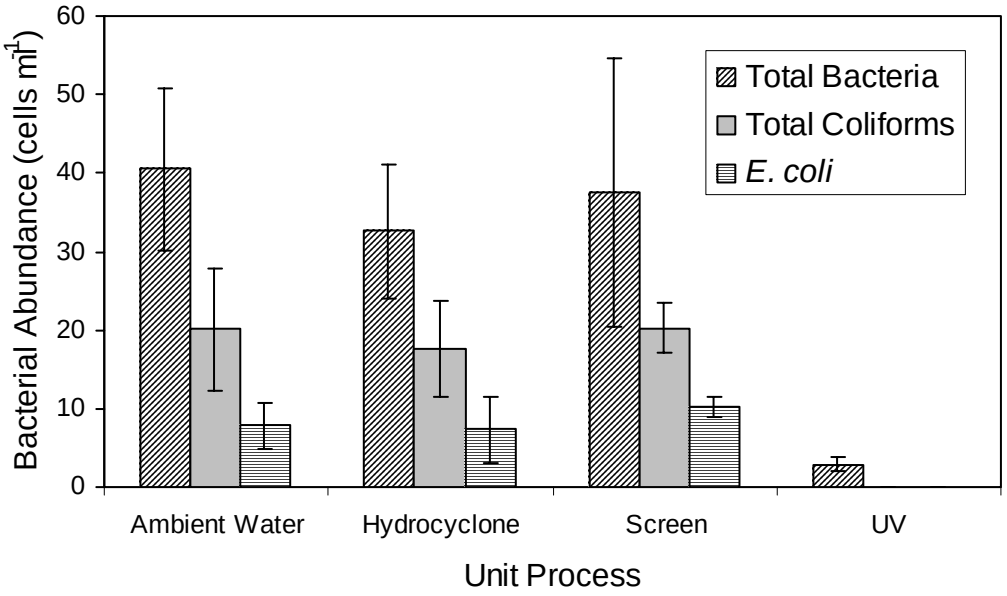


Figure 4. Effect of hydrocyclone, screen and UV on bacterial abundance at Time = 0 h.

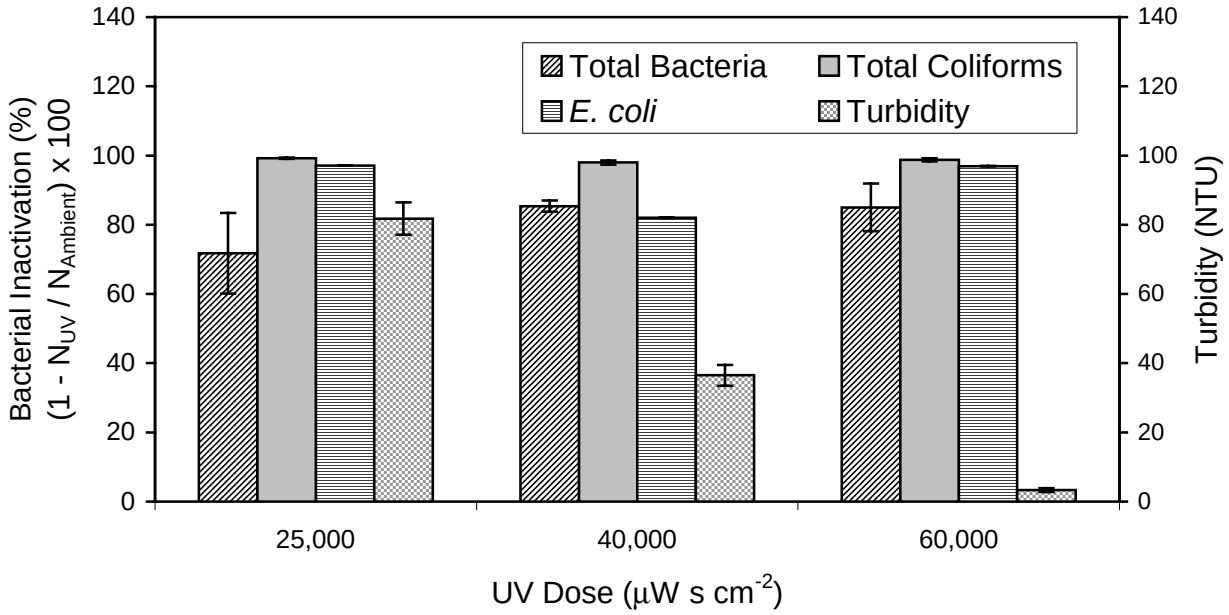


Figure 5. Inactivation of bacteria at different UV doses caused by varying turbidities.

Table 5. Bacterial concentrations in ambient untreated water and after different UV dose treatments caused by varying turbidities.

Response variable	High dose				Medium dose				Low dose			
	$N_{Ambient}$		N_{UV}		$N_{Ambient}$		N_{UV}		$N_{Ambient}$		N_{UV}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Bacteria	41 (4-67)	10	2.9 (1-6.3)	0.9	43 (12-8)	8.7	8.5 (4.3-15)	1.8	130 (21-310)	48	0.2	0.1
Total Coliforms	20 (3-48)	7.8	0.1 (0.1)	0	8.9 (2.2-22)	3.3	0.1 (0.1-0.2)	0.1	16 (4.8-27)	3.2	0.1 (0.1)	0
E. coli	7.9 (1.4-18)	2.9	0.1 (0.1)	0	1.9 (0.3-6)	0.9	0.1 (0.1)	0	6.1 (1.6-15)	2.1	0.1 (0.1)	0

High dose: 60,000 µW s cm^{-2} at ambient turbidity (1 to 5 NTU)

Medium dose: 40,000 µW s cm^{-2} at 30 NTU turbidity

Low dose: 25,000 µW s cm^{-2} at 80 NTU turbidity

$N_{Ambient}$: concentration (cells ml^{-1}) in ambient untreated water

N_{UV}: concentration (cells ml^{-1}) in UV treated water after screen or hydrocyclone treatment

S.E.: ± 1 standard error

Total Bacteria: total cultivable heterotrophic bacteria

Range shown in brackets

that utilized in this experiment (> 60,000 µW·s cm^{-2}) to guarantee reliable and predictable removal of microorganisms. Clearly, the regrowth issue observed here will mean that the required UV dose would have to be excessively high to ensure that all organisms are permanently inactivated.

The effect of ballast water treatment schemes was evaluated against phytoplankton abundance in ambient seawater. Figure 7 shows phytoplankton biomass as a function of each unit process. In our tests, chlorophyll *a* was utilized as a monitor of phytoplankton biomass. In addition, phaeophytin, which is considered to be a principal breakdown product of chlorophyll *a*, was also recorded. It was anticipated that if inactivation of phytoplankton occurred, then perhaps it could be monitored by the occurrence of a degradation product of chlorophyll. This figure shows that the levels of chlorophyll *a* were not affected by physical separation processes but were slightly affected by UV treatment. It was found, however, that phaeophytin remained constant throughout all analyses, thereby negating its use in our studies as an indicator of chlorophyll breakdown.

All of the data relating to phytoplankton chlorophyll were grouped in order to compare UV effects as a function of introduced turbidity. As discussed above, this was possible because no effects on phytoplankton abundance due to physical separation treatment were observed. Figure 8 and Table 7 show a summary of these data, and it can be seen that no trends are apparent with

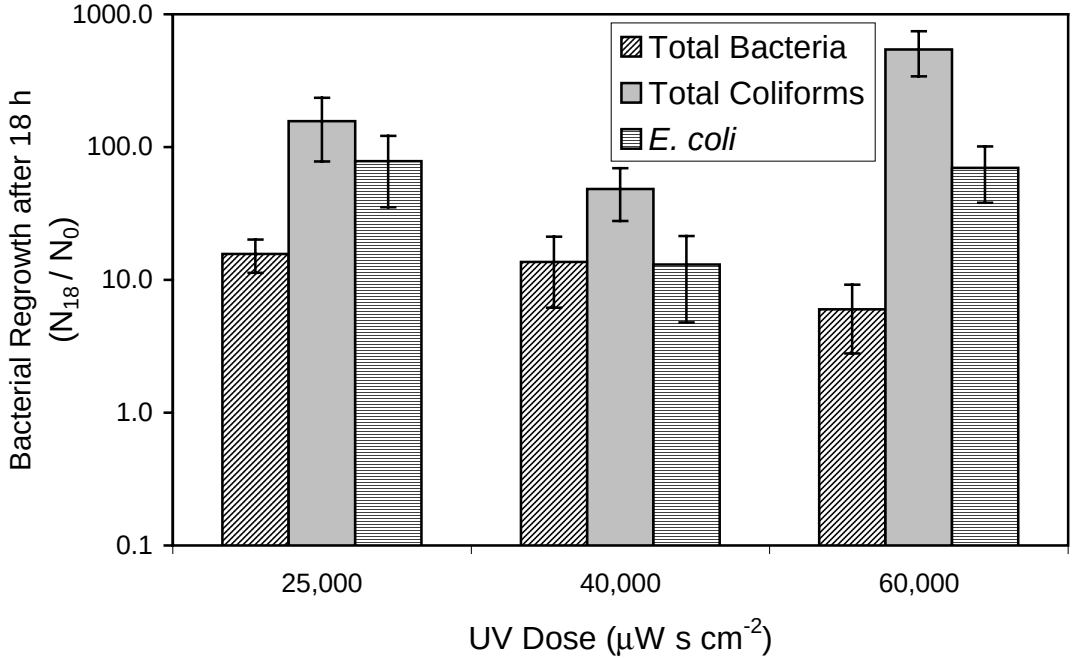


Figure 6. Bacterial regrowth in seawater held for 18 h after UV treatment.

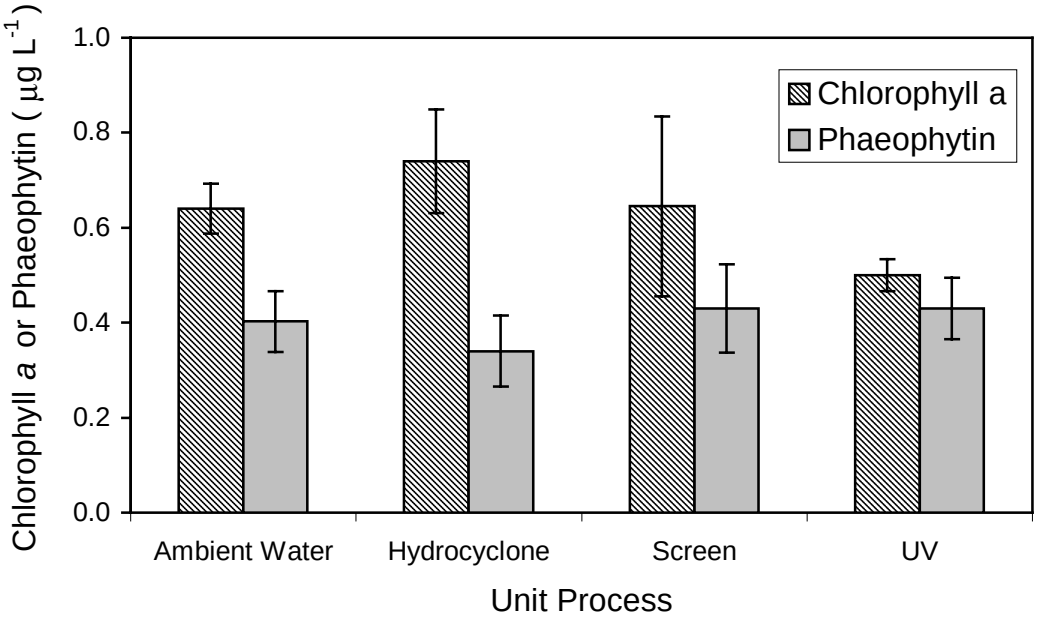


Figure 7. Effect of hydrocyclone, screen and UV unit processes on total phytoplankton population.

Table 6. Bacterial concentrations in UV treated water 0 h and 18 h after treatment.

Response variable	High dose				Medium dose				Low dose			
	N_0		N_{18}		N_0		N_{18}		N_0		N_{18}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Bacteria	3.3 (1-6.3)	1	20 (1-67)	12	9.9 (4.7-15)	2.4	150 (29-460)	100	18 (3.3-36)	5.8	240 (9-450)	61
Total Coliforms	0.1 (0.1)	0	12 (3.8-33)	5.4	0.1 (0.1-0.2)	0.1	6.2 (0.7-17)	5.6	0.4 (1-7.3)	1.2	2.3 (0.6-5)	0.7
E. coli	0.1 (0.1)	0	0.7 (0.1-1.9)	0.3	0.1 (0.1)	0	0.1 (0.1-0.4)	0.1	0.1 (0.1)	0	0.8 (0.1-2.7)	0.4

High dose: 60,000 μW s cm^{-2} at ambient turbidity (1 to 5 NTU)

Medium dose: 40,000 μW s cm^{-2} at 30 NTU turbidity

Low dose: 25,000 μW s cm^{-2} at 80 NTU turbidity

N_0: concentration (cells ml^{-1}) after screen or hydrocyclone and UV treatment at Time = 0 h

N_{18}: concentration (cells ml^{-1}) after screen or hydrocyclone and UV treatment at Time = 18 h

S.E.: ± 1 standard error

Total Bacteria: total cultivable heterotrophic bacteria

respect to destruction of chlorophyll *a* with either increasing or decreasing UV treatment. Therefore, it appears that the use of ultraviolet radiation for destroying phytoplankton in ballast water would not be an efficient or predictable process, and probably would not be successful in producing ballast water free of phytoplankton. It should also be noted however, that while chlorophyll *a* is effectively a monitor of biomass, it may not be a sensitive measure of inactivation of photosynthetic activity due to UV treatment. It is anticipated that even if a vegetative cell is inactivated, it takes some time (hours to days) before the chlorophyll present in the cell is reduced or disappears altogether.

The use of chlorophyll *a* as an indicator of phytoplankton viability can be further addressed by looking at the fate of the photosynthetic pigment after UV treatment. Figure 9 and Table 8 show the relationship of chlorophyll *a* and phaeophytin 18 h after irradiation as a function of the UV dose delivered. Once again, the amount of phaeophytin pigments does not seem to change appreciably with dose. Thus, a chlorophyll breakdown pigment such as the phaeophytin measured here is probably not a sensitive measure of injury to the phytoplankton population. The figure also shows that the remaining chlorophyll in samples 18 h after radiation (measured as the ratio of chlorophyll present 18 h after treatment normalized by the amount present immediately after treatment) is approximately half of that at the time of radiation. This ratio of pigments appears to be insensitive to the dose delivered. It does however, indicate that some loss in chlorophyll is observed upon incubation, and therefore, possible injury to phytopigments occurred due to UV treatment. Longer grow-out analyses would be required to determine the total extent of phytoplankton biomass reduction achievable by UV treatment.

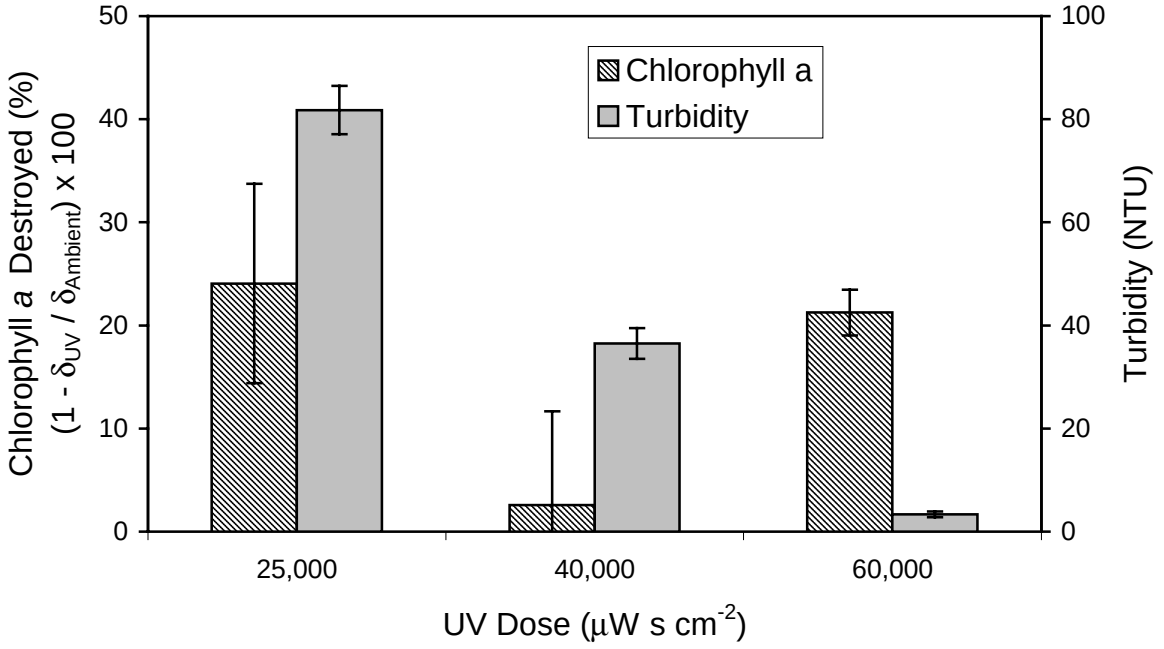


Figure 8. Effect of different UV doses caused by varying turbidities on total phytoplankton population.

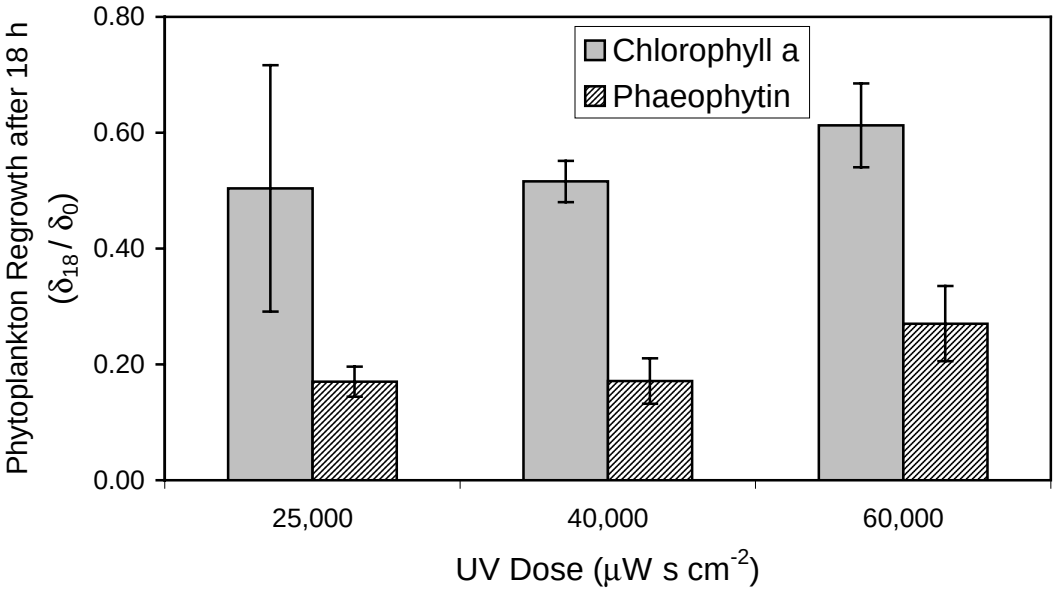


Figure 9. Regrowth of total phytoplankton in seawater held for 18 h after UV treatment.

Table 7. Phytoplankton biomass in ambient untreated water and after different UV dose treatments caused by varying turbidities.

Response variable	High dose				Medium dose				Low dose			
	$\delta_{Ambient}$		δ_{UV}		$\delta_{Ambient}$		δ_{UV}		$\delta_{Ambient}$		δ_{UV}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Chlorophyll *a*	0.6 (0.4-0.8)	0.1	0.5 (0.4-0.6)	0.1	0.8 (0.4-1)	0.2	0.8 (0.4-2)	0.2	0.5 (0.3-0.7)	0.1	0.4 (0.1-0.6)	0.1
Total Phaeophytin	0.4 (0.2-0.6)	0.1	0.4 (0.2-0.6)	0.1	0.4 (0.1-0.9)	0.1	0.5 (0.1-0.8)	0.1	0.4 (0.2-0.5)	0.1	0.4 (0.3-0.5)	0.1

High dose: 60,000 µW s cm^{-2} at ambient turbidity (1 to 5 NTU)

Medium dose: 40,000 µW s cm^{-2} at 30 NTU turbidity

Low dose: 25,000 µW s cm^{-2} at 80 NTU turbidity

$\delta_{Ambient}$: concentration (µg L^{-1}) in ambient untreated water

δ_{UV}: concentration (µg L^{-1}) in UV treated water after screen or hydrocyclone treatment

S.E.: ± 1 standard error

Range shown in brackets

Table 8. Phytoplankton biomass in UV treated water 0 h and 18 h after treatment.

Response variable	High dose				Medium dose				Low dose			
	δ_0		δ_{18}		δ_0		δ_{18}		δ_0		δ_{18}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Chlorophyll *a*	0.5 (0.4-0.6)	0.1	0.3 (0.2-0.4)	0.1	0.6 (0.4-0.8)	0.1	0.3 (0.3-0.4)	0.1	0.4 (0.1-0.6)	0.1	0.1 (0.1-0.2)	0.1
Total Phaeophytin	0.4 (0.2-0.6)	0.1	0.4 (0.1-0.2)	0.1	0.4 (0.4-0.8)	0.1	0.5 (0.1-0.2)	0.1	0.4 (0.3-0.5)	0.1	0.4 (0.1-0.2)	0.1

High dose: 60,000 µW s cm^{-2} at ambient turbidity (1 to 5 NTU)

Medium dose: 40,000 µW s cm^{-2} at 30 NTU turbidity

Low dose: 25,000 µW s cm^{-2} at 80 NTU turbidity

δ_0: concentration (µg L^{-1}) after screen or hydrocyclone and UV treatment at Time = 0 h

δ_{18}: concentration (µg L^{-1}) after screen or hydrocyclone and UV treatment at Time = 18 h

S.E.: ± 1 standard error

Range shown in brackets

In addition to bacteria and phytoplankton, zooplankton assemblages were also monitored as a function of treatment type and added suspended solids. The zooplankton species observed during our studies were those most commonly found in the seawater samples during the test period. These included gastropod larvae, copepod nauplii, members of the Order Harpacticoida and representative species of the Genera *Oithona*, *Paracalanus*, and *Acartia*. Other organisms were observed, but were not present consistently, or in large enough numbers to be analyzed statistically. Qualitative microscopic observations of untreated vs. separation treated (screen or hydrocyclone), or separation plus UV treated samples, made immediately following sample collection, revealed no apparent loss of vitality by any zooplankton groups observed in the samples. That is, virtually all samples contained lively specimens, regardless of the treatment method. These observations suggest that the UV treatment utilized here was not sufficient to kill the mesozooplankton groups present.

Figures 10 and 11 show the relative concentrations of the selected zooplankton species used to monitor treatment efficiency. Figure 10 shows a typical concentration of both gastropod and bivalve larvae observed during testing, and it can be seen that the average occurrence of larvae is relatively small, averaging less than 3 organisms per liter. Figure 11 shows the density of different copepod groups under various test conditions. These data, as well as those for Figure 10 were grouped to include tests run with the hydrocyclone and with the self-cleaning screen. Figure 11 shows that a relatively large number of Copepoda nauplii are present (> 10 per liter) in the test water while other copepods appear in much lower numbers, quite often less than 1 to 2 organisms per liter. Figure 12 shows the removal efficiency of both gastropod and bivalve larvae by screen and hydrocyclonic treatment. The data are also given in Tables 9 and 10. The screen consistently removes well over 90 per cent of the larvae, regardless of the level of suspended solids in the water. Because most of the larvae analyzed during this test program were substantially larger than 50 μm, it was expected that the majority of the larvae would be removed. In contrast, the overall removal of larvae by the hydrocyclone was extremely low. However, there appears to be an increasing trend in removal efficiency as a function of suspended solids. It is possible that the added kaolinite clay was aggregating larvae together into a large enough mass to be affected by the hydrocyclone and therefore removed. In fact, visual observation of samples after addition of clay showed that many of the larvae were indeed aggregated together. However, even with a large load of suspended solids, with turbidities to close to 90 NTU, less than 40 per cent of the larvae were removed by the hydrocyclone. Considering that the majority of invasions recorded to date have occurred because of transport of bivalve and gastropod larvae, removal of these particular components of the zooplankton becomes especially important. From our studies, it is shown that the screen operating at 50 μm can effectively remove the majority of any of the larvae present in natural water systems.

Figure 13 shows a comparison of the two unit processes (screen versus hydrocyclone) for removal of the copepods monitored in this study. The data are also given in Tables 9 and 10. As with the larvae, the screen appears to remove a substantial amount of copepods while the hydrocyclone is ineffective at removing them. The copepods investigated in this study were significantly smaller than the gastropod and bivalve larvae shown in Figure 12, however, a substantial percentage was still removed by the 50 μm screen. Figure 13 also shows that there was variation in the final number of organisms after hydrocyclone treatment. However, removal

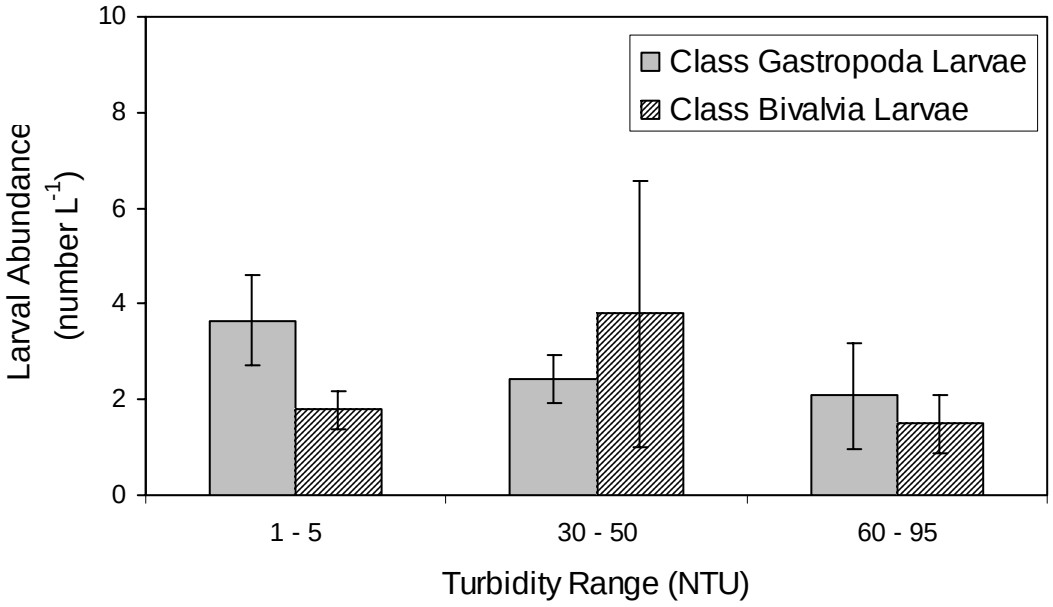


Figure 10. Ambient concentrations of total gastropod and bivalve larvae at different turbidity levels.

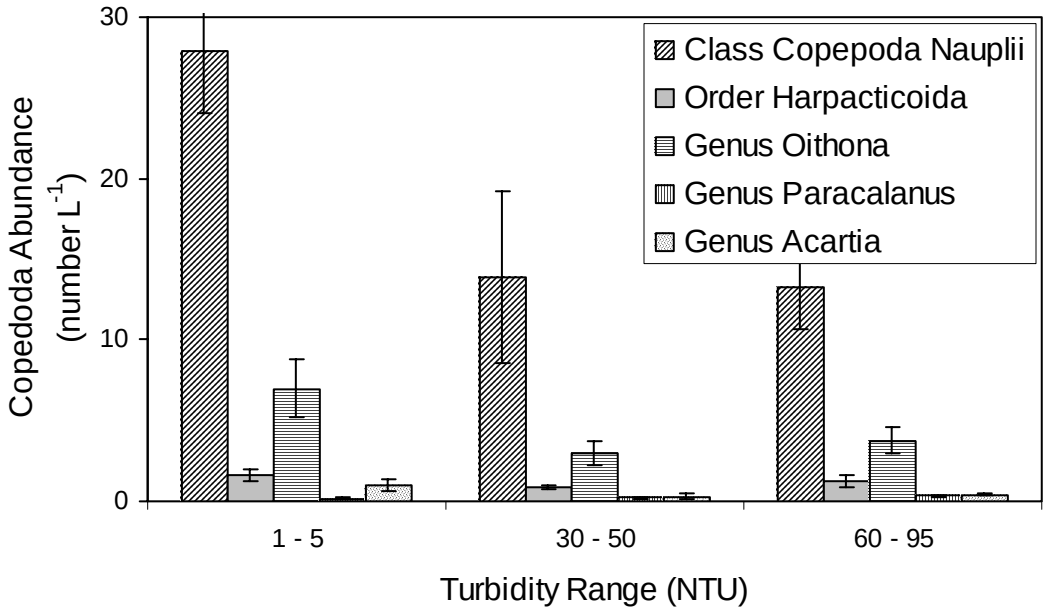


Figure 11. Ambient concentrations of copepods at different turbidity levels.

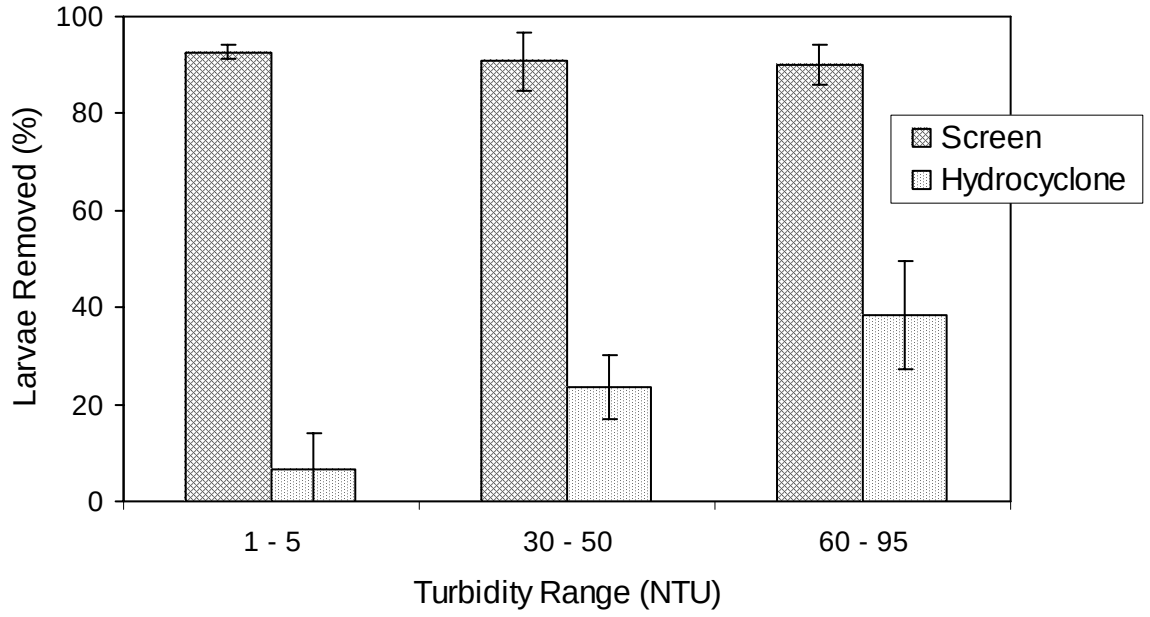


Figure 12. Physical removal of gastropod and bivalve larvae by screen and hydrocyclone at different turbidity levels.

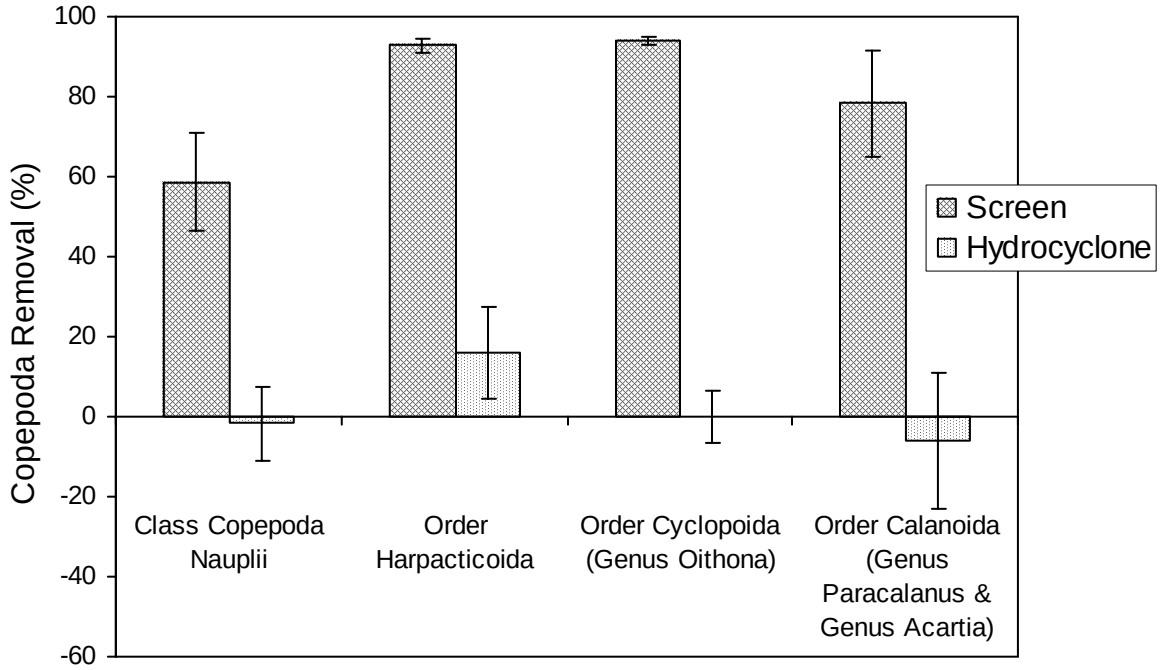


Figure 13. Physical removal of copepods by screen vs. hydrocyclone.

Table 9. Zooplankton concentrations in ambient untreated water and after screen treatment at different turbidities.

Response variable	Low turbidity				Medium turbidity				High turbidity			
	$N_{Ambient}$		N_{Screen}		$N_{Ambient}$		N_{Screen}		$N_{Ambient}$		N_{Screen}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Acartia spp. (Order Calanoida)	0.8 (0.2-1.2)	0.3	0 (0)	0	0.3 (0.1-0.5)	0.2	0.1 (0.1-0.2)	0.1	0.1 (0-0.3)	0.1	0.1 (0-0.1)	0.1
Paracalanus spp. (Order Calanoida)	0.1 (0-0.3)	0.1	0 (0)	0	0.2 (0-0.4)	0.2	0 (0)	0	0.3 (0.1-0.7)	0.2	0 (0)	0
Oithona spp. (Order Cyclopoida)	8.1 (2.6-15)	3.5	0.3 (0.1-0.7)	0.2	2.6 (2.4-2.8)	0.2	0.2 (0.1-0.3)	0.1	3.4 (2.5-5.1)	1.5	3 (0.1-0.3)	0.8
Order Harpacticoida	2 (1-3.3)	0.7	0.1 (0.1-0.2)	0.1	0.8 (0.5-1)	0.3	0.1 (0.1-0.2)	0.1	1.4 (0.7-2.2)	0.4	0.1 (0-0.4)	0.1
Class Copepoda Nauplii	30 (18-39)	6	9.3 (3.3-15)	3.3	17 (5-28)	12	8.3 (5.6-11)	2.7	10 (7.5-13)	2.7	2.9 (1.1-4.6)	1.7
Class Gastropoda Larvae	4 (2-7.8)	1.8	0.2 (0.1-0.3)	0.1	2.8 (2-3.7)	0.8	0.3 (0.1-0.5)	0.2	1.3 (0.8-2.2)	0.5	0.1 (0.1-0.2)	0.1
Class Bivalvia Larvae	1.5 (0.1-2.6)	0.7	0.2 (0.1-0.3)	0.1	7.4 (0.4-15)	7.1	0.4 (0-0.9)	0.4	1.7 (0.9-2.5)	0.5	0.2 (0-0.5)	0.2

Low turbidity: 1 to 5 NTU
Medium turbidity: 30 NTU
High turbidity: 80 NTU

$N_{Ambient}$: concentration (number L^{-1}) in ambient untreated water

N_{Screen}: concentration (number L^{-1}) after screen treatment

S.E.: ± 1 standard error

Range shown in brackets

Table 10. Zooplankton concentrations in ambient untreated water and after hydrocyclone treatment at different turbidities.

	Low turbidity				Medium turbidity				High turbidity			
	$N_{Ambient}$		$N_{Hydrocyclone}$		$N_{Ambient}$		$N_{Hydrocyclone}$		$N_{Ambient}$		$N_{Hydrocyclone}$	
Response variable	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
spp. (Order Calanoida)	1.3 (0.2-2.8)	0.8	1.1 (0.3-2.5)	0.7	0.3 (0-0.8)	0.3	0.2 (0.1-0.4)	0.1	0.4 (0.2-0.6)	0.1	0.4 (0.1-0.6)	0.2
anus spp. (Order Calanoida)	0.2 (0.1-0.6)	0.2	0.3 (0-0.7)	0.2	0.2 (0.1-0.3)	0.1	0.3 (0.1-0.4)	0.1	0.3 (0.2-0.6)	0.1	0.3 (0.1-0.4)	0.1
spp. (Order Cyclopoida)	5.8 (3.2-8.6)	1.5	4.7 (3.4-6.9)	1.1	3.2 (1.5-5.7)	1.3	3.3 (1.8-5.9)	1.3	4.1 (1.7-6.9)	1.5	4.8 (1.6-7.9)	1.8
arpacticoida	1.2 (0.8-1.7)	0.3	0.9 (0.3-1.2)	0.3	0.9 (0.7-1.2)	0.1	0.9 (0.3-1.5)	0.3	1.1 (0.5-2.3)	0.6	0.8 (0.3-1.5)	0.4
opepoda Nauplii	30 (19-50)	9	23 (18-31)	4	12 (5.6-26)	6.8	14 (5.1-28)	7.3	12 (6.4-23)	5.5	14 (5-31)	8.4
astropoda Larvae	3.3 (2-5.2)	0.1	3.3 (1.6-6.3)	1.5	2.2 (0.9-3.4)	0.7	1.5 (0.7-2.4)	0.5	2.8 (0.4-7.5)	2.3	1.7 (0.3-4.4)	1.4
valvia La☆ae	2.1 (1.2-2.7)	0.5	1.9 (1.3-2.4)	0.3	1.4 (0-4.1)	1.4	1 (0-2.9)	1	1.3 (0.1-4)	1.3	1.2 (0.1-3.4)	1.1

bidity: 1 to 5 NTU
turbidity: 30 NTU
bidity: 80 NTU
concentration (number L^{-1}) in ambient untreated water
lone: concentration (number L^{-1}) after hydrocyclone treatment
standard error
nown in brackets

of organisms via the hydrocyclone is clearly small, regardless of the variability in the data shown here.

In addition to bacterial, phytoplankton, and zooplankton monitoring in the test protocol, biochemical analyses were also utilized to evaluate their usefulness in monitoring treatment efficiency. One procedure was the measurement of protein, which is an indicator of the standing biomass present at any time. Protein was monitored in two different components of seawater, i.e., those organisms larger than 35 μm and those organisms smaller than 35 μm. The > 35 μm fraction represents the zooplankton groups and other larger organisms (macrobiota), while the remainder represents principally the microbiota. Figure 14 shows the concentration of protein as a function of treatment process for both size fractions. There was substantially more protein in the microbiota fraction versus the macrobiota fraction. For that component of the biota larger than 35 μm, there appears to be some removal through the screen and hydrocyclone, but no change after UV treatment. This is to be expected, as differences in protein should only be observed if biomass is removed, as would be the case after hydrocyclone or screen treatment. It is not expected that UV treatment would cause a change in protein.

While the measurement of biomass can be achieved in many ways, the determination of organism viability or activity is a more critical monitor. Other than for organisms such as bacteria, where plate growth studies can be easily undertaken, it is difficult to determine for viability of larger organisms on a routine basis. In order to address this issue, an attempt was made to evaluate the use of ATP as a monitor of organism activity. ATP is perhaps the single most important macromolecule used by live organisms in carrying out their biochemical processes. Organisms were separated into two size fractions (< 35 μm or > 35 μm) and analyzed for the amount of ATP present per unit volume. Figure 15 shows the summary of these experiments. For ambient seawater, the unit concentration of ATP was substantially higher in the < 35 μm size fraction. It can also be seen that this number decreases through the physical separation processes of both hydrocyclone and screen, and remains constant after UV treatment. These results indicate that ATP levels decreased because of biomass removal; therefore ATP does reflect changes that occurred due to treatment. In the case of the microbiota fraction ATP, it can be seen that these levels remain constant through the physical separation process, which would also be expected as organisms smaller than 35 μm would not be removed. However, the small fraction ATP remained high after UV treatment, even though all other monitors (e.g. bacterial counts) indicated that the fraction was significantly reduced due to UV treatment.

It appears from the preliminary data presented here indicate that ATP may be a useful tool for monitoring activity of the biomass as affected by treatment processes. However, more work is required to refine this process, so that reliable and reproducible data can be collected.

Table 11 shows the statistical evaluation of the various biological and biochemical indices to the screen or screen plus UV treatment, as well as any impact on the unit processes due to dose effects. As mentioned previously for Table 2, the main variable used in statistical tests was *d*, the difference in the amount of the response variable before and after treatment by a given unit process. Statistical significance for a unit process indicated that mean *d* was different from zero, and statistical significance for dose impact indicated that mean *d* differed among UV doses affected by different water color levels (low, medium, high color). The dose impact indicates

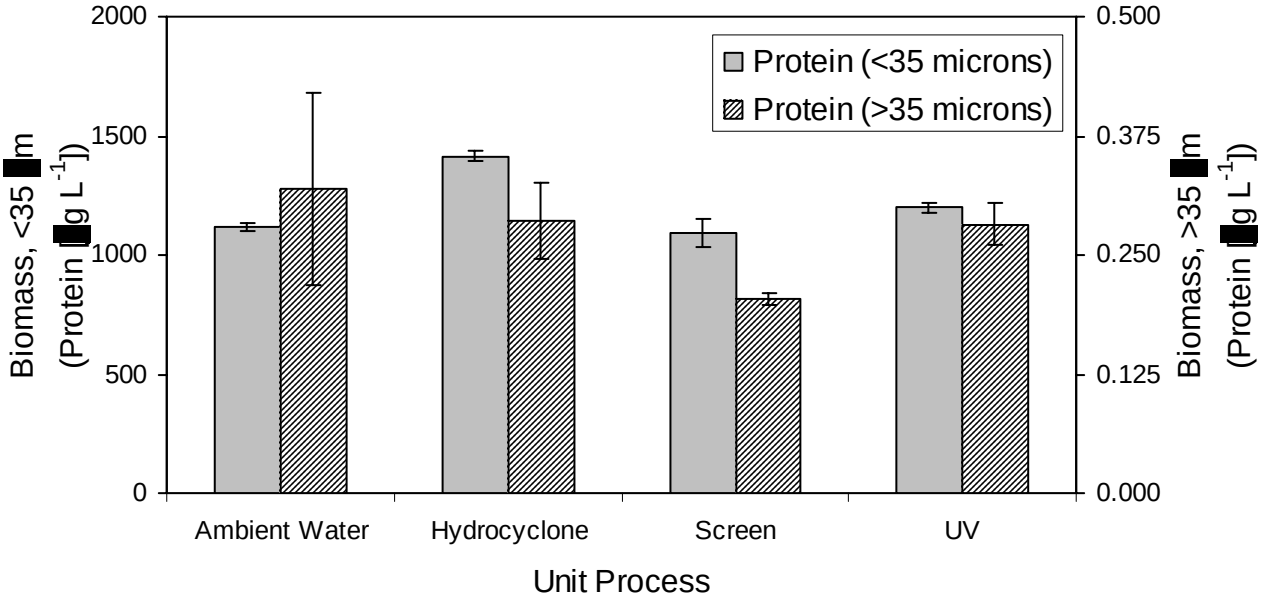


Figure 14. Effect of hydrocyclone, screen and UV treatment processes on biomass.

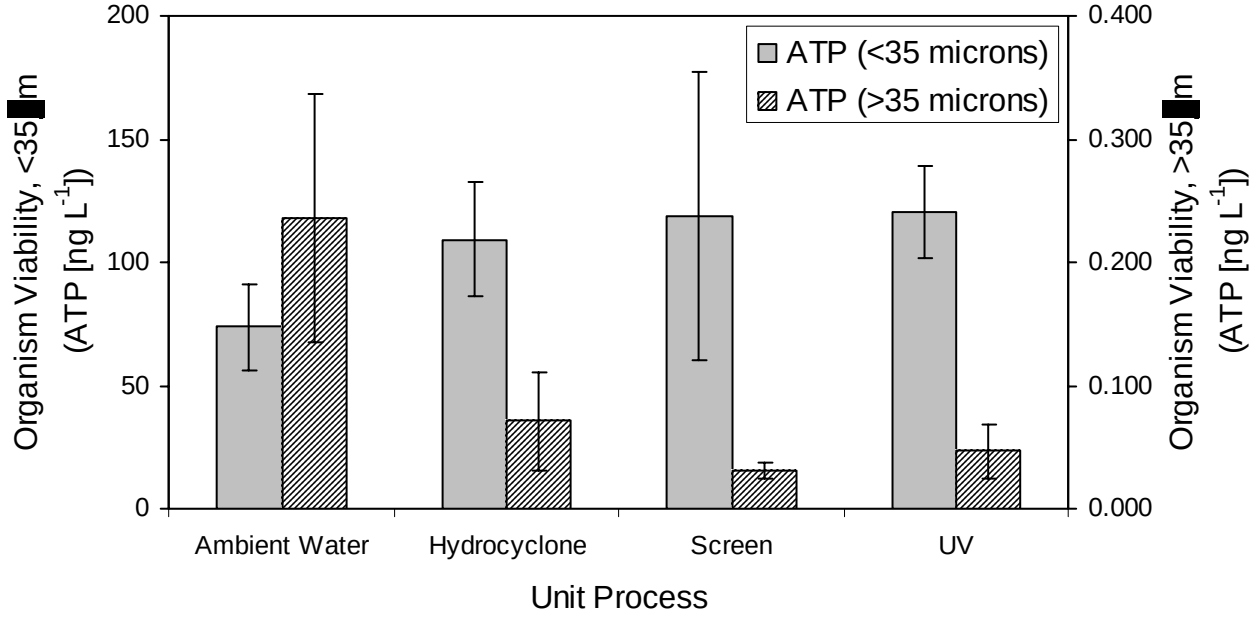


Figure 15. Effect of hydrocyclone, screen and UV treatment processes on organism viability as measured by ATP.

whether there were differences in the response variable among the UV dose treatments. Again, enumeration of zooplankton was omitted in those test conditions where no discernable effect was anticipated (after UV treatment and/or 24 h exposure to light after 6 d dark incubation)

As observed in the first set of experiments with different turbidity conditions, the 50 μm screen was again effective in removing the various zooplankton groups (*Acartia, Paracalanus, Oithona,* Harpacticoida, Copepoda nauplii, gastropod and bivalve larvae). There was significant reduction of all zooplankton groups monitored ($p < 0.001$). Similarly, UV treatment at high doses (60,000 $\mu W \cdot s\ cm^{-2}$) was capable of significantly ($p < 0.05$) reducing bacterial abundance (total cultivable heterotrophic bacteria, total coliforms, *E. coli*). At a UV dose of 45,000 $\mu W \cdot s\ cm^{-2}$, total coliforms and *E. coli* numbers were significantly lowered ($p < 0.05$). This is consistent with results from the first experiment in that UV dose in excess of 25,000 $\mu W \cdot s\ cm^{-2}$ was sufficient to inactivate naturally occurring bacteria in seawater. Bacterial numbers were not significantly reduced however, when UV treatment was applied at a relatively low dose (10,000 $\mu W \cdot s\ cm^{-2}$). In contrast to the prior experiments with turbidity, phytoplankton biomass, as monitored by chlorophyll *a* and phaeophytin, was noted to be significantly removed by the screen ($p < 0.05$). ATP, protein and ATP per protein values in the large (> 35 μm) fraction were significantly reduced after screen treatment ($p > 0.01$). This is consistent with other observations, that is, there was significant removal of all zooplankton groups monitored. In addition, ATP, protein and ATP per protein values in the small (< 35 μm) fraction were not affected by screen treatment. This is also as expected, as there was no significant removal of any of the bacteria by the screen. ATP, protein and ATP per protein values in the small fraction however, did not significantly decrease after UV treatment at high and medium doses (60,000 and 45,000 $\mu W \cdot s\ cm^{-2}$, respectively) even though significant reduction in components of the small fraction were noted. As discussed previously, ATP and protein protocols and measurements will need more refinement in order to be considered robust and dependable indicators of overall organism biomass and viability.

Table 12 shows the statistical evaluation for the 50 μm screen and UV unit processes including the 6 d dark incubation period after treatment. Effects of a 6 d storage period were noted in both ambient and screened water samples for bacteria (total cultivable heterotrophic bacteria, total coliforms and *E. coli*) and phytoplankton biomass (chlorophyll *a* and phaeophytin). Significant decreases in bacterial numbers and phytoplankton biomass were observed ($p > 0.001$). This in contrast to the prior set of experiments when samples were held for 18 h, and significant regrowth was noted for all bacterial indices. Significant changes were also observed in some of the zooplankton indices monitored. There were significantly higher numbers of *Acartia* and *Paracalanus* species in both ambient and screened water samples held for 6 d in the dark ($p < 0.001$). No significant changes in number were noted in *Oithona* species and harpacticoid copepods in ambient water samples held for 6 d, however, significantly higher abundances were observed in the screened water samples ($p < 0.001$). In contrast, significantly lower counts of copepod nauplii were enumerated in both ambient and screened water samples stored for 6 d. Naupliar or juvenile stages of copepods are difficult to visually classify to the genus level, thus they were identified to the class level. It appears that during the 6 d incubation, copepod nauplii matured to adult stages, which allowed visual identification to the species and order level. No significant changes were noted in gastropod or bivalve larvae abundances after 6 d incubation in ambient or screened water samples. It may be that the significant decreases in bacteria and

phytoplankton observed above may be due to natural causes, or perhaps to grazing by the maturing copepods. For the most part, ATP, protein, and ATP per protein values did not reflect the significant changes noted in the other response variables.

Table 13 shows the statistical evaluation of data collected for the 50 µm screen and UV unit processes including the 24 h incubation period in the light following 6 d dark storage. Over the entire test scenario, there was either no effect of 24 h light incubation, or significant decreases in bacterial abundance or phytoplankton biomass ($p < 0.01$), indicating that regrowth of bacteria or phytoplankton did not occur.

Figure 16 is a graphic representation of the treatment effects on the bacterial populations monitored in ambient seawater at 0 d. Similar to that observed previously, concentrations of total cultivable heterotrophic bacteria and coliforms remained unchanged from the ambient seawater through the 50 µm screen. In addition, the UV system facilitated significant removal of bacteria, with essentially all of the coliforms (total and *E. coli*) being removed to below detection levels (10 organisms per 100 ml), and only a small residual of total cultivable heterotrophic bacteria remaining in the water at ambient color. These data also show that bacterial abundance in test waters of this facility was similar to that enumerated previously (approximately 10^1 cells ml^{-1}).

It was expected that water color would affect UV dose and thus, UV efficiency. Figure 17 and Table 14 show the effect of UV dose on bacterial inactivation. UV doses of 45,000 and 60,000 $\mu W \cdot s\ cm^{-2}$ were sufficient to reduce the number of total cultivable heterotrophic bacteria and coliforms. This result is consistent with that observed previously in the turbidity experiments, where UV doses of 25,000 $\mu W \cdot s\ cm^{-2}$ and higher significantly reduced bacterial populations in natural water systems. A UV dose of 10,000 $\mu W \cdot s\ cm^{-2}$ (representing approximately 20 mg l^{-1} humic material in water) however, was not sufficient to completely inactivate any of the bacterial groups tested.

Figure 18 shows the concentration of total heterotrophic cultivable bacteria, total coliforms and *E. coli* in ambient and "screen plus UV" treated water samples stored for 6 d in the dark and 6 d in the dark, followed by 24 h exposure to natural light. In contrast to previous results, which showed significant bacterial regrowth in samples held for 18 h after UV treatment, no regrowth was observed in samples held for 6 d. It should be noted that bacterial abundances decreased in all incubated samples, regardless of UV dose. This indicates that factors other than UV treatment were responsible for the decline in bacterial numbers over time.

The effects of screen and UV unit processes were evaluated against phytoplankton abundance in ambient seawater. Figure 19 shows that levels of chlorophyll *a* decreased slightly in water samples passed through the screen, indicating some removal of phytoplankton biomass by the screen, but there was no change in biomass after UV treatment. Similar to the results noted previously, phaeophytin remained constant throughout all analyses, thereby negating its use in our studies as an indicator of chlorophyll breakdown.

As observed previously, UV dose did not affect the amount of phytoplankton biomass destroyed (Figure 20 and Table 15). Again, it appears that UV radiation alone would not be an effective

Table 11. Response variables vs. efficiency of screen and UV unit processes (Time = 0 h).

Response variable	Unit process: Screen — Significant reduction	Unit process: Screen — Dose impact	Unit process: Screen + UV — Significant reduction: High dose	Unit process: Screen + UV — Significant reduction: Medium dose	Unit process: Screen + UV — Significant reduction: Low dose
Total Bacteria	N	N	***	N	N
Total Coliforms	N	N	*	*	N
E. coli	N	*	*	**	N
Total Chlorophyll *a*	***	N	N	**	N
Total Phaeophytin	*	*	N	*	N
Acartia spp. (Order Calanoida)	***	ND	ND	ND	ND
Paracalanus spp. (Order Calanoida)	***	ND	ND	ND	ND
Oithona spp. (Order Cyclopoida)	***	ND	ND	ND	ND
Order Harpacticoida	***	ND	ND	ND	ND
Class Copepoda Nauplii	***	ND	ND	ND	ND
Class Gastropoda Larvae	***	ND	ND	ND	ND
Class Bivalvia Larvae	***	ND	ND	ND	ND
ATP (> 35 µm)	***	N	N	N	N
ATP (< 35 µm)	N	N	N	N	N
Protein (> 35 µm)	***	N	N	N	N
Protein (< 35 µm)	N	N	N	N	N
ATP/Protein (> 35 µm)	**	N	N	N	N
ATP/Protein (< 35 µm)	N	N	N	N	N

High dose: 60,000 µW s cm^{-2}

Medium dose: 45,000 µW s cm^{-2}

Low dose: 10,000 µW s cm^{-2}

*: significant at < 0.05

**: significant at < 0.01

***: significant at < 0.001

N: no effect

ND: not determined

Total Bacteria: total cultivable heterotrophic bacteria

Table 12. Response variables vs. efficiency of screen with UV system (Time = 0 d vs. 6 d dark).

	Unit process					
	Ambient water	Screen		Screen + UV		
	Change after 6 d dark	Change after 6 d dark	Dose impact	Change after 6 d dark		
Response variable				High dose	Medium dose	Low dose
Total Bacteria	***, D	***, D	N	N	N	N
Total Coliforms	***, D	***, D	**	N	N	**, D
E. coli	***, D	***, D	**	N	N	**, D
Total Chlorophyll *a*	***, D	***, D	N	**, D	**, D	*, D
Total Phaeophytin	***, D	***, D	N	**, D	**, D	N
Acartia spp. (Order Calanoida)	***, I	***, I	ND	ND	ND	ND
Paracalanus spp. (Order Calanoida)	***, I	***, I	ND	ND	ND	ND
Oithona spp. (Order Cyclopoida)	N	***, I	ND	ND	ND	ND
Order Harpacticoida	N	***, I	ND	ND	ND	ND
Class Copepoda Nauplii	***, D	***, D	ND	ND	ND	ND
Class Gastropoda Larvae	N	N	ND	ND	ND	ND
Class Bivalvia Larvae	N	N	ND	ND	ND	ND
ATP (> 35 µm)	**, D	N	N	N	N	*, D
ATP (< 35 µm)	N	N	**	*, D	N	N
Protein (> 35 µm)	N	N	N	N	N	N
Protein (< 35 µm)	N	N	N	N	N	N
ATP/Protein (> 35 µm)	*, D	N	N	N	N	N
ATP/Protein (< 35 µm)	N	N	N	N	N	N

High dose: 60,000 µW s cm^{-2}; Medium dose: 45,000 µW s cm^{-2}; Low dose: 10,000 µW s cm^{-2}
*: significant at < 0.05
**: significant at < 0.01
***: significant at < 0.001
I: increase
D: decrease
N: no effect
ND: not determined
Total Bacteria: total cultivable heterotrophic bacteria

Table 13. Response variables vs. efficiency of screen with UV system (Time = 6 d dark vs. 6 d dark + 24 h light).

	Unit process					
	Ambient water	Screen		Screen + UV		
	Change from 6 d dark to 6 d dark + 24 h light	Change from 6 d dark to 6 d dark + 24 h light	Dose impact	Change from 6 d dark to 6 d dark + 24 h light		
Response variable				High dose	Medium dose	Low dose
Total Bacteria	**, D	N	N	N	N	N
Total Coliforms	***, D	**, D	N	N	N	N
E. coli	***, D	**, D	N	N	N	N
Total Chlorophyll *a*	N	N	N	N	**, D	N
Total Phaeophytin	***, D	***, D	N	N	*, D	N
Acartia spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
Paracalanus spp. (Order Calanoida)	ND	ND	ND	ND	ND	ND
Oithona spp. (Order Cyclopoida)	ND	ND	ND	ND	ND	ND
Order Harpacticoida	ND	ND	ND	ND	ND	ND
Class Copepoda Nauplii	ND	ND	ND	ND	ND	ND
Class Gastropoda Larvae	ND	ND	ND	ND	ND	ND
Class Bivalvia Larvae	ND	ND	ND	ND	ND	ND
ATP (> 35 µm)	*, D	ND	ND	ND	ND	ND
ATP (< 35 µm)	N	N	N	N	N	N
Protein (> 35 µm)	N	N	N	N	N	N
Protein (< 35 µm)	N	N	N	N	N	N
ATP/Protein (> 35 µm)	N	N	N	N	N	N
ATP/Protein (< 35 µm)	N	*, D	N	N	N	N

High dose: 60,000 µW s cm^{-2}; Medium dose: 45,000 µW s cm^{-2}; Low dose: 10,000 µW s cm^{-2}
*: significant at < 0.05
**: significant at < 0.01
***: significant at < 0.001
I: increase
D: decrease
N: no effect
ND: not determined
Total Bacteria: total cultivable heterotrophic bacteria

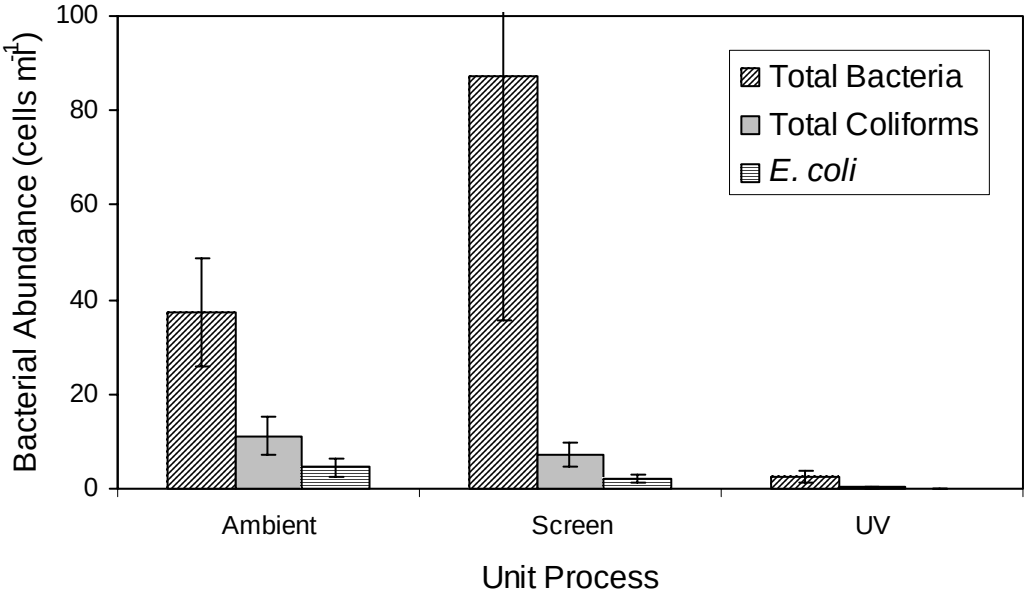


Figure 16. Effect of screen and UV unit processes on bacterial abundance in ambient seawater at Time = 0 d.

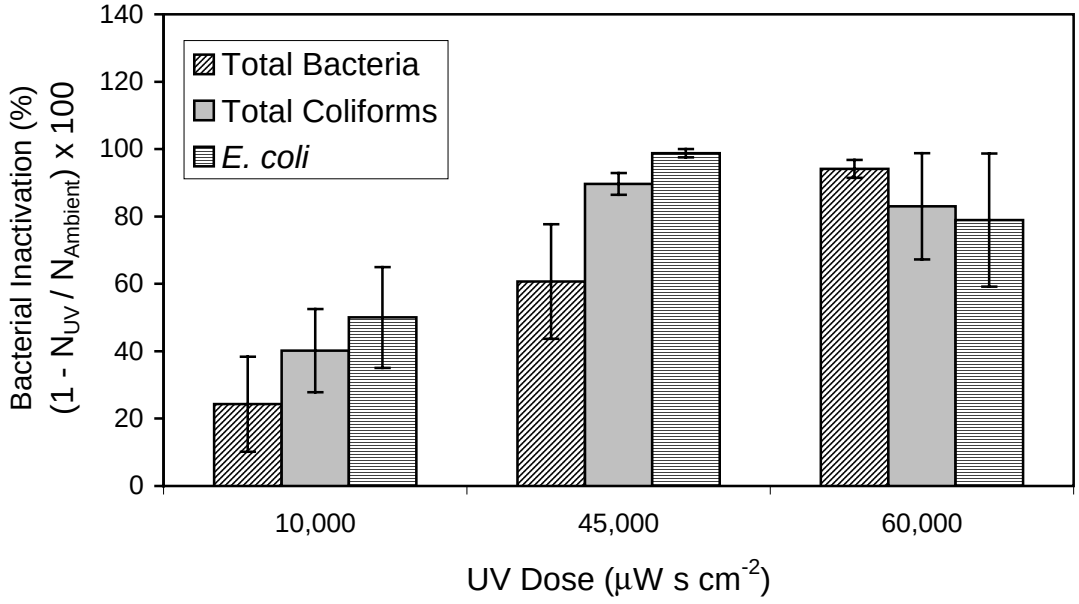


Figure 17. Inactivation of bacteria at different UV doses.

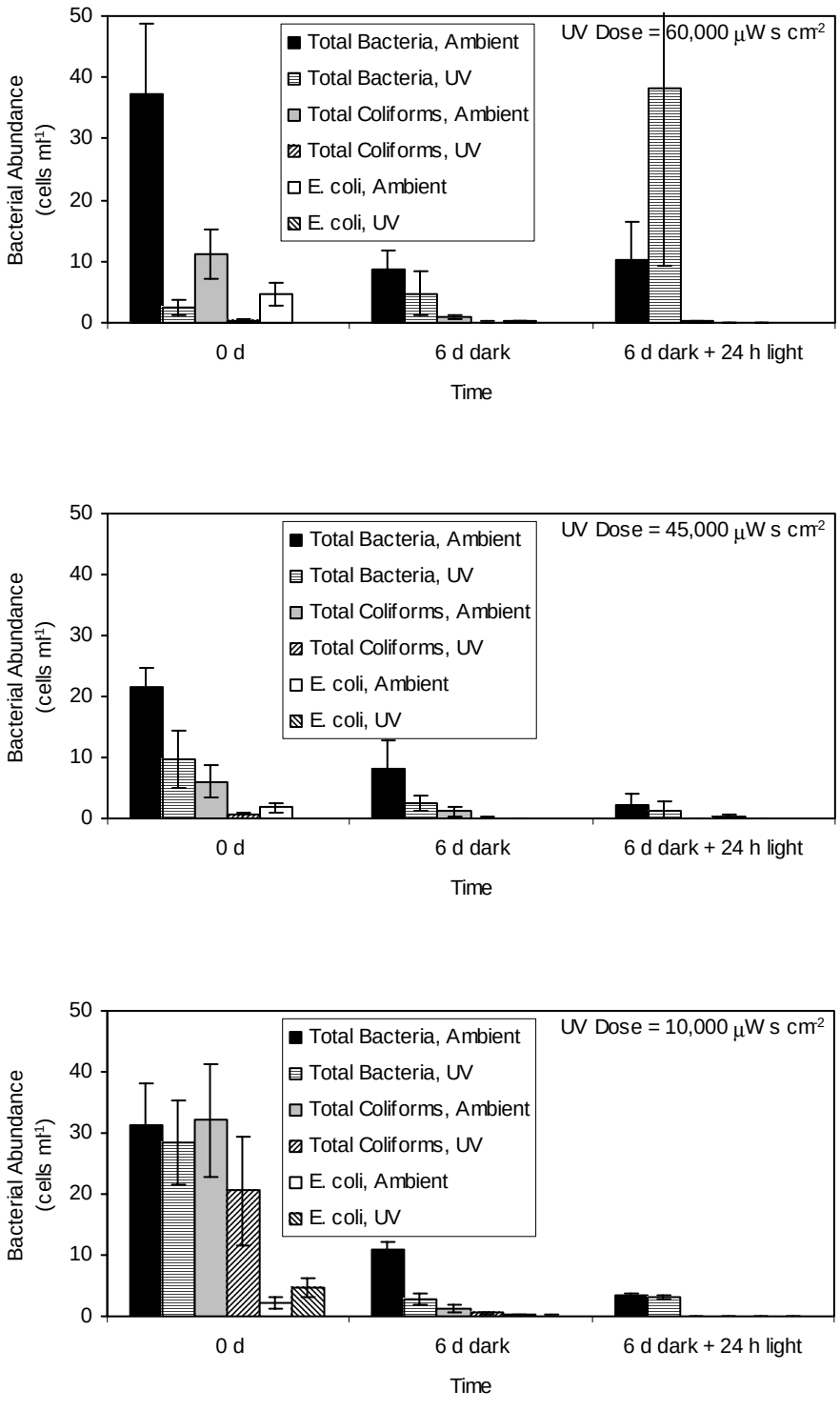


Figure 18. Bacterial concentration after UV treatment and 6 d dark storage, and 6 d dark + 24 h light storage.

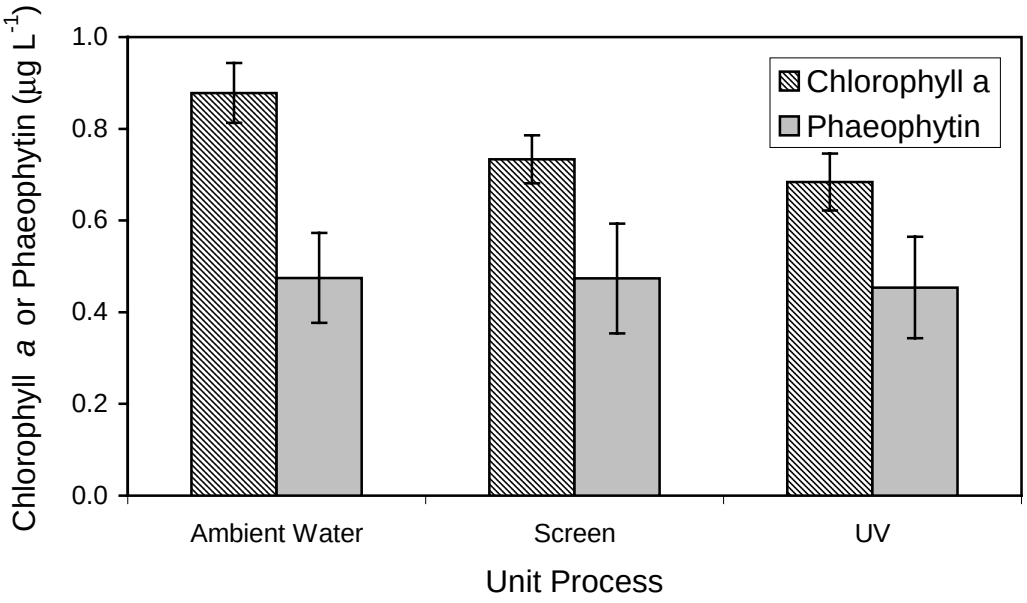


Figure 19. Effect of screen and UV unit processes on total phytoplankton population at ambient water color.

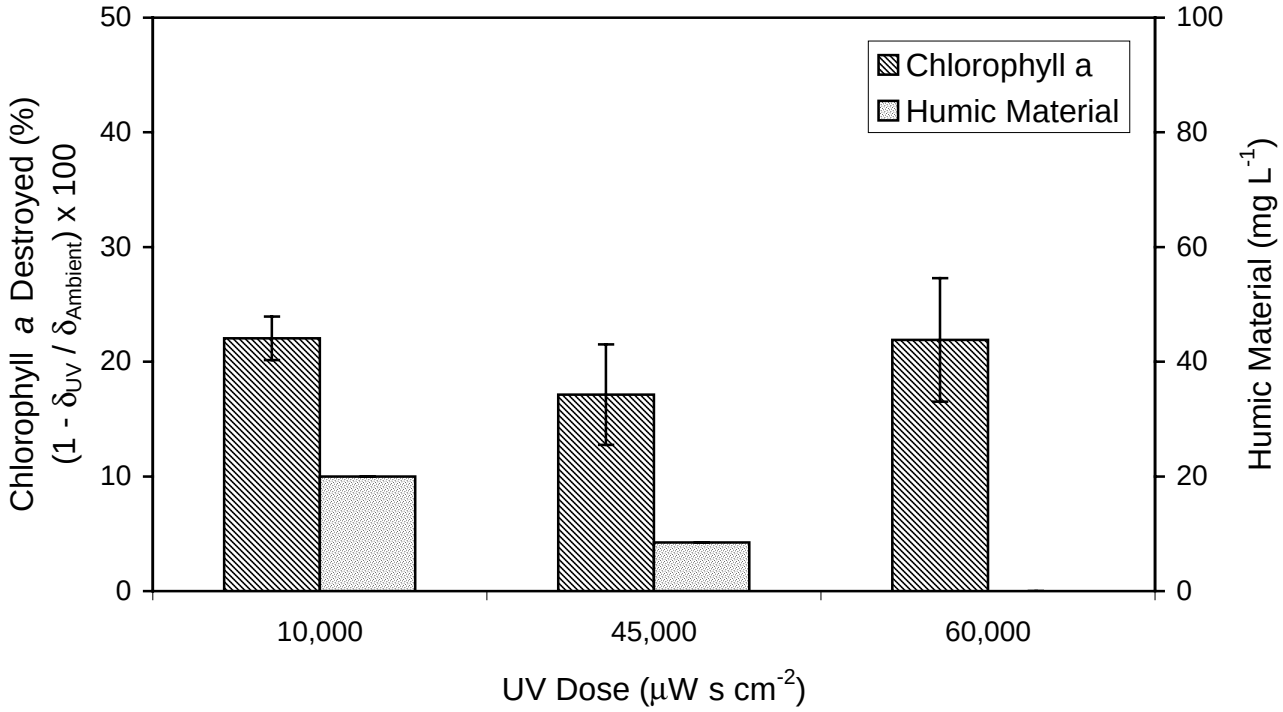


Figure 20. Effect of UV dose on total phytoplankton population at Time = 0 d.

Table 14. Bacterial concentrations in ambient untreated water and after different UV dose treatments.

Response variable	High dose				Medium dose				Low dose			
	$N_{Ambient}$		N_{UV}		$N_{Ambient}$		N_{UV}		$N_{Ambient}$		N_{UV}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Bacteria	49 (28-77)	9.9	3 (0-7)	1.2	22 (13-27)	2.9	9.6 (1-27)	4.6	32 (2.7-90)	15	28 (2.7-84)	15
Total Coliforms	11 (0.5-22)	4.1	0.3 (0.1-1.1)	0.2	6 (2.9-17)	2.6	0.6 (0.1-1.5)	0.3	32 (15-66)	9.2	21 (5.8-56)	9
E. coli	4.6 (0.1-9.4)	1.9	0.1 (0.1-0.3)	0.1	1.7 (0.5-4.4)	0.7	2 (0.1-10)	2	4.8 (0.9-9.7)	1.6	2.3 (0.3-5.2)	0.9

High dose: 60,000 µW s cm^{-2}

Medium dose: 45,000 µW s cm^{-2}

Low dose: 10,000 µW s cm^{-2}

$N_{Ambient}$: concentration (cells ml^{-1}) in ambient untreated water

N_{UV}: concentration (cells ml^{-1}) in UV treated water after screen treatment

S.E.: ± 1 standard error

Total Bacteria: total cultivable heterotrophic bacteria

Range shown in brackets

Table 15. Phytoplankton biomass in ambient untreated water and after different UV dose treatments.

Response variable	High dose				Medium dose				Low dose			
	$\delta_{Ambient}$		δ_{UV}		$\delta_{Ambient}$		δ_{UV}		$\delta_{Ambient}$		δ_{UV}	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Total Chlorophyll *a*	3.5 (2.9-4.1)	0.3	2.7 (2.2-3.2)	0.2	4.1 (1.9-5.9)	0.8	3.3 (1.9-4.7)	0.6	1.9 (1.6-2.4)	0.2	1.5 (1.2-1.8)	0.1
Total Phaeophytin	1.9 (0.9-2.8)	0.4	1.8 (0.8-2.8)	0.4	1.5 (0.7-2)	0.3	1.3 (0.4-1.8)	0.3	0.5 (0-1.2)	0.2	0.6 (0.1-1.7)	0.3

High dose: 60,000 µW s cm^{-2}

Medium dose: 45,000 µW s cm^{-2}

Low dose: 10,000 µW s cm^{-2}

$\delta_{Ambient}$: concentration (µg L^{-1}) in ambient untreated water

δ_{UV}: concentration (µg L^{-1}) in UV treated water after screen treatment

S.E.: ± 1 standard error

Range shown in brackets

process for inactivating phytoplankton in ballast water. It should be noted again however, that even if a vegetative cell is inactivated, it takes some time (hours to days) before the chlorophyll present in the cell is reduced or disappears altogether.

In contrast to the results reported for phytoplankton inactivation 18 h after UV treatment, significant decreases in chlorophyll *a* were noted in samples held for 6 d in the dark after treatment (Figure 21). Because there was no effect of UV dose on the amount of photosynthetic pigments, all the data were grouped together for this analysis. There was no change in chlorophyll *a* in samples held for a further 24 h in natural light. Phaeophytin, a chlorophyll breakdown pigment, again did not increase with a decrease in chlorophyll *a* as expected. Even after the 6 d dark incubation, when phaeophytin levels were anticipated to increase as a result of chlorophyll breakdown, no such changes were noted.

Figure 22 shows that on average, 60 to 90 per cent of the zooplanktonic organisms monitored were consistently removed by the 50 µm screen, with 80 to 95 per cent removal occurring in five of the seven taxonomic groups enumerated (data given in Table 16). This is consistent with results observed previously for the screen, which exhibited much higher removal efficiencies of zooplankton than the hydrocyclone. Removals of 60 to 65 per cent were observed in the two remaining taxonomic groups: Class Copepoda nauplii and Genus *Acartia* copepods. Copepod nauplii are smaller than the adult stages, thus it was expected that the per cent removed by the screen is lower than that for the adults. In the water color experiments, the abundance of *Acartia* in the source water was less than in the turbidity experiments, and the data is based on fewer observations.

Significant changes in concentrations of various copepod groups were noted in samples passed through the 50 µm screen, and incubated for 6 d in the dark. Figure 23 shows that copepod nauplii concentrations decreased in the 6 d samples, with concomitant increases in harpacticoid copepods and, *Oithona, Paracalanus* and *Acartia* species. It is evident that during the 6 d storage period, the naupliar stages of the copepods matured to adult stages, which could subsequently be visually identified to a more accurate taxonomic level. Grazing by these maturing copepods may also be responsible for the decrease in chlorophyll *a* and phaeophytin levels observed in the 6 d incubation described above.

The effectiveness of removal of particles by the media filter is presented by particle size distributions from 8 µm to 240 µm. It should be noted that the Multisizer 3® particle counter does not differentiate shapes, and converts particle volume to an equivalent spherical volume. The samples were run three times each for 60 s, and a representative figure for the three replicates is shown. In addition, an overlay of the figures obtained allows comparison of the results graphically and indicates the main differences due to the loading rate changes. Before media tests were conducted, a control run was done. Figure 24 shows the distribution of particles in replicate runs of seawater passed through the filter without media. No difference amongst particle size distributions is evident.

Figures 25 to 29 show the particle size distributions for each of the different media tested at various loading rates. Although the particle size distribution in the ambient seawater was different for every experiment, the results suggest that there is a noticeable decrease of particles

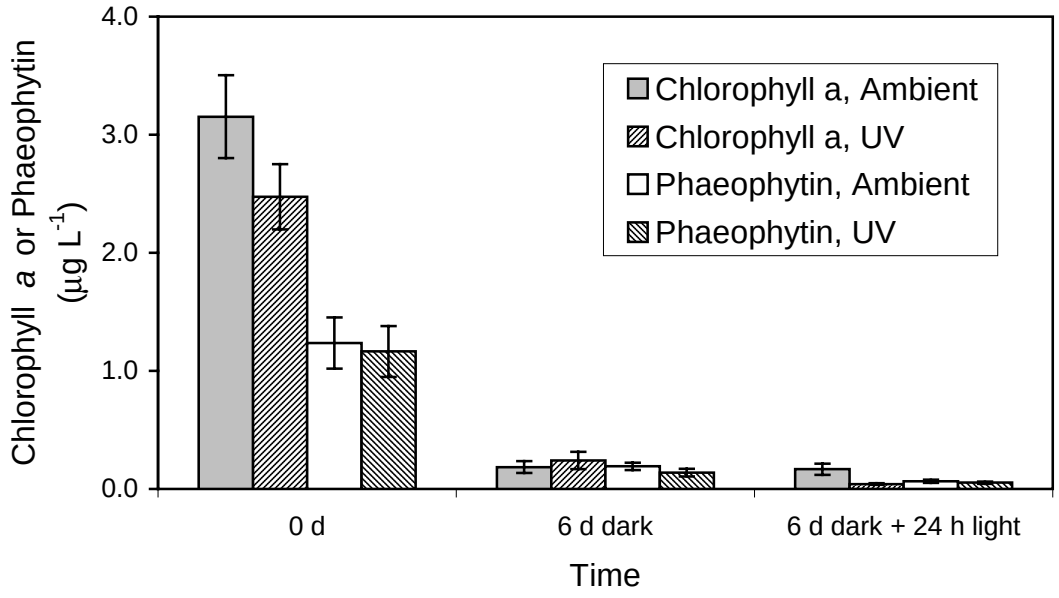


Figure 21. Phytoplankton biomass after UV treatment and 6 d dark storage and 6 d dark + 24 h light storage.

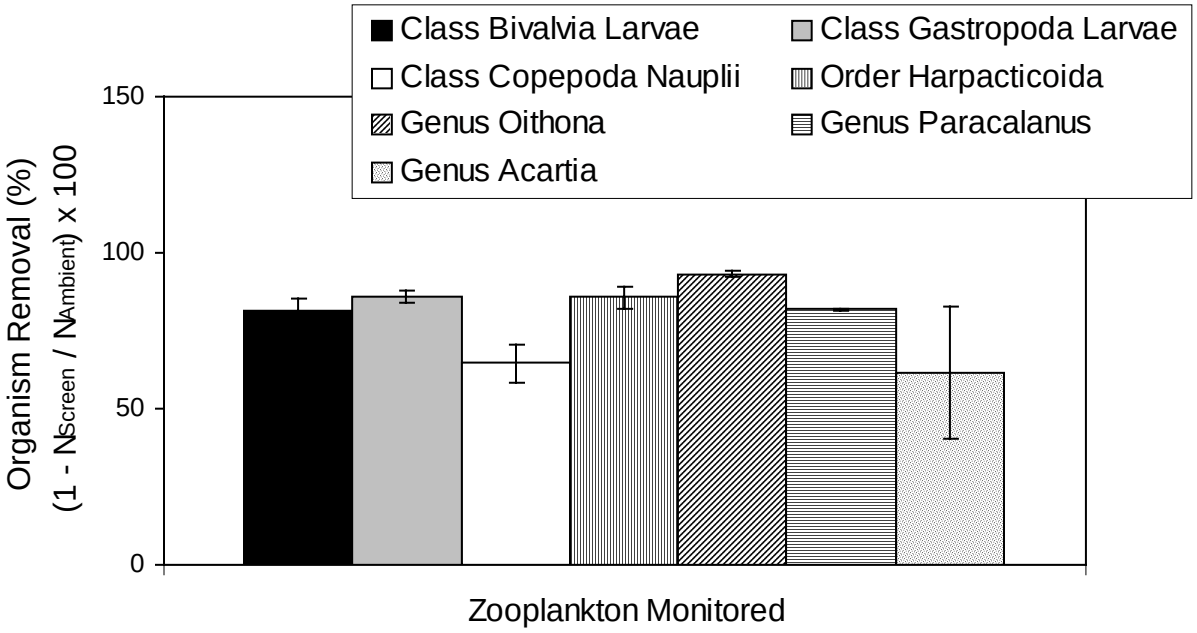


Figure 22. Physical removal of selected zooplankton by the screen.

Table 16. Zooplankton concentrations in ambient untreated water and after screen treatment.

Response variable	$N_{Ambient}$		N_{Screen}	
	Mean	S.E.	Mean	S.E.
Acartia spp. (Order Calanoida)	0.3 (0-0.9)	0.1	0.1 (0-0.2)	0.1
Paracalanus spp. (Order Calanoida)	0.5 (0-1.6)	0.1	0.1 (0-0.3)	0.1
Oithona spp. (Order Cyclopoida)	7.1 (1.3-41)	2.6	0.3 (0-0.9)	0.1
Order Harpacticoida	3.4 (0.2-36)	2.4	0.1 (0-0.4)	0.1
Class Copepoda Nauplii	20 (6-36)	2.2	7.2 (0.8-19)	1.3
Class Gastropoda Larvae	1.9 (0.2-7.1)	0.5	0.3 (0-0.7)	0.1
Class Bivalvia Larvae	4.6 (0.1-11)	1.0	0.6 (0-1.3)	0.1

$N_{Ambient}$: concentration (number L^{-1}) in ambient untreated water

N_{Screen}: concentration (number L^{-1}) after screen treatment

S.E.: ± 1 standard error

Range shown in brackets

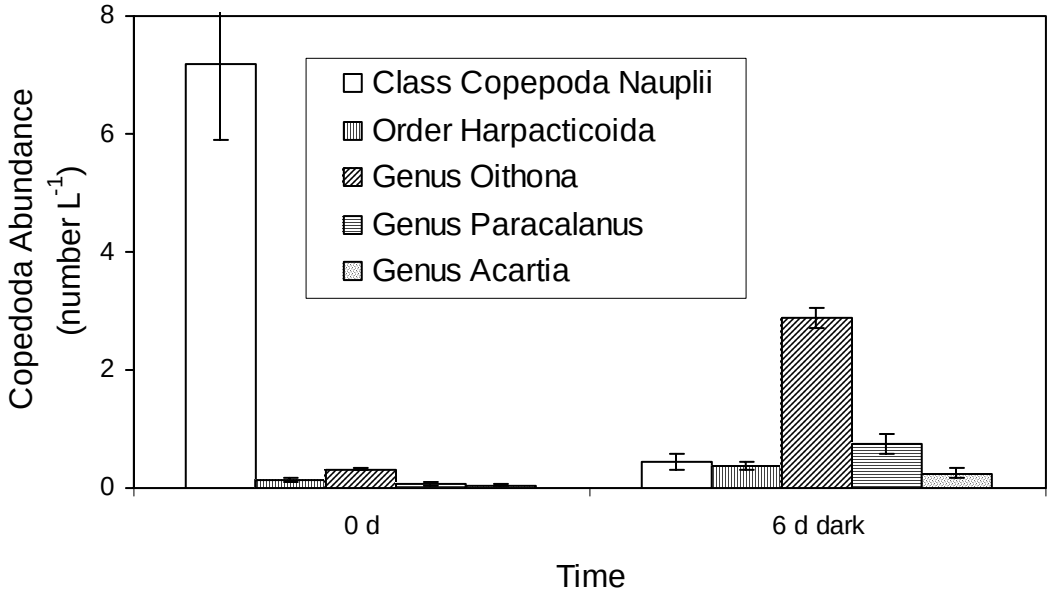


Figure 23. Copepod abundance in screened samples held for 6 d in the dark.

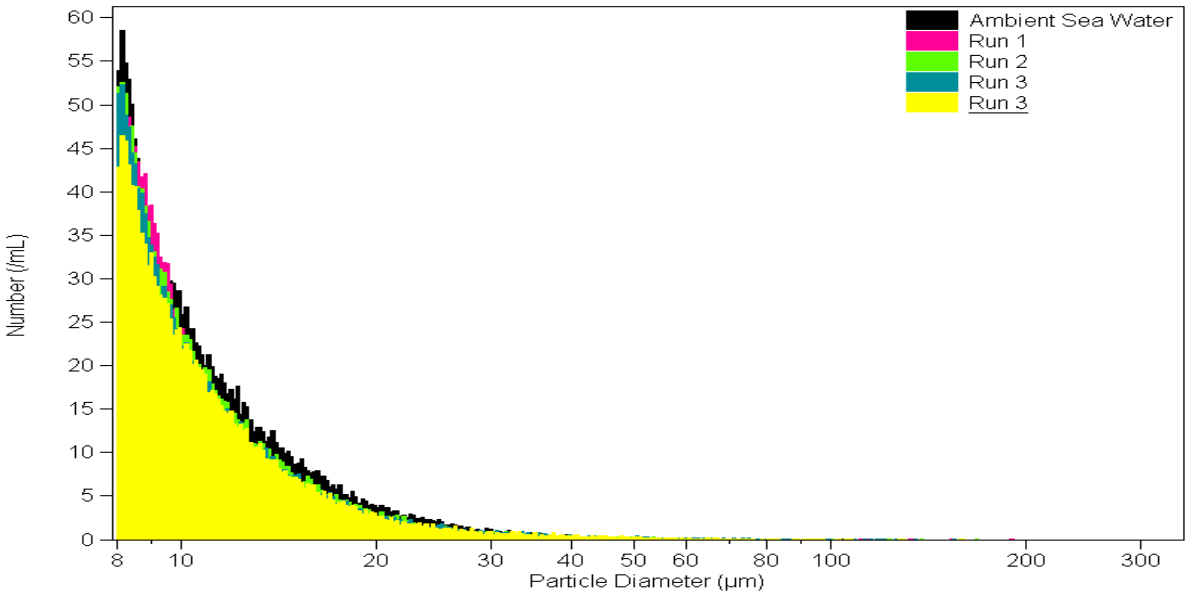


Figure 24. Particle size distributions in seawater passed through the media filter without any media.

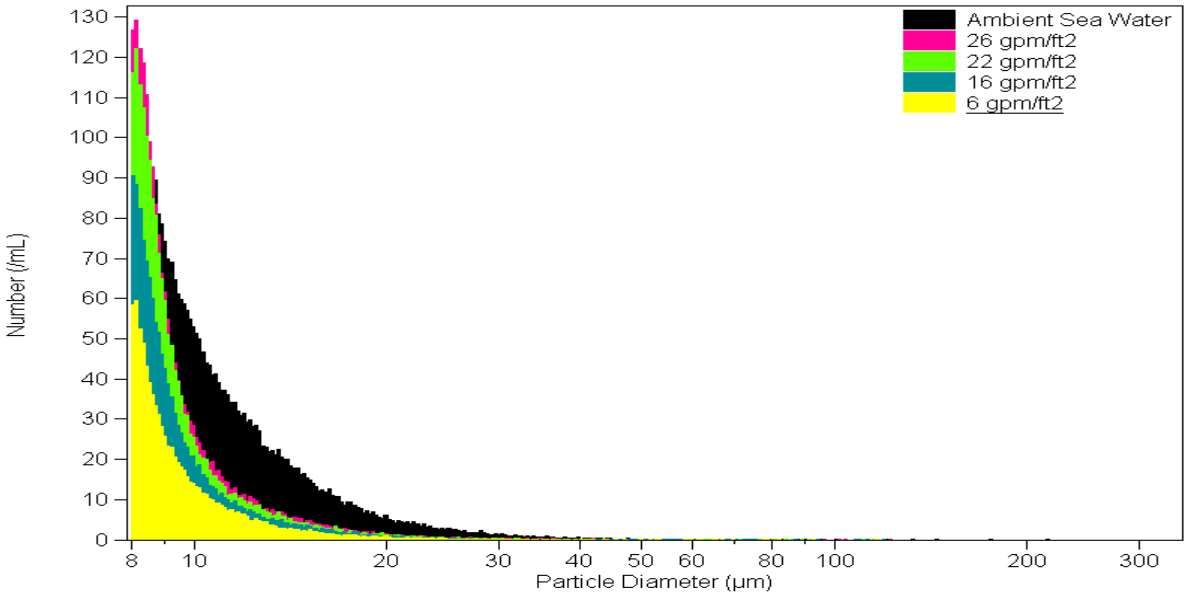


Figure 25. Particle size distributions in seawater passed through Silica Sand (0.80 to 1.2 mm) at various loading rates.

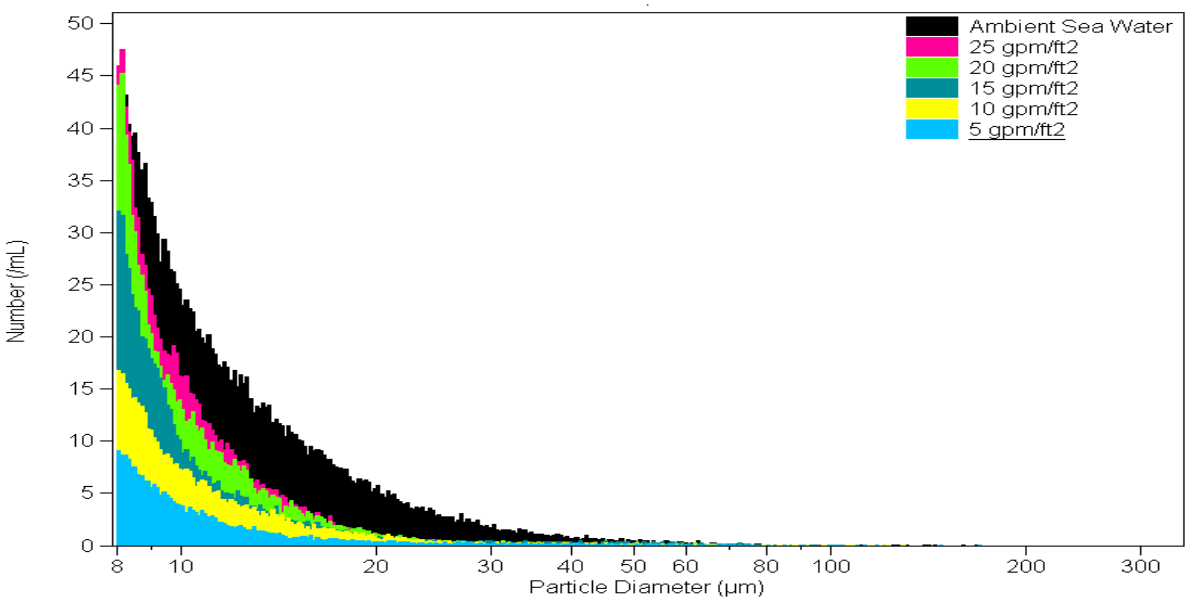


Figure 26. Particle size distributions in seawater passed through Silica Sand (0.45 to 0.55 mm) at various loading rates.

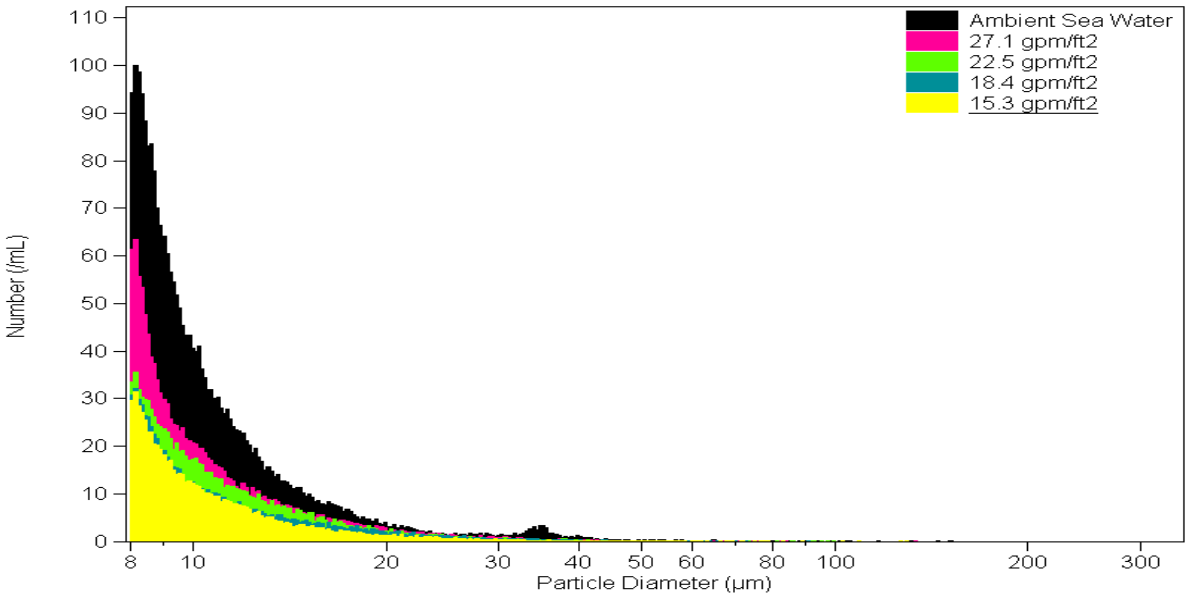


Figure 27. Particle size distributions in seawater passed through Anthracite (0.95 to 1.05 mm) at various loading rates.

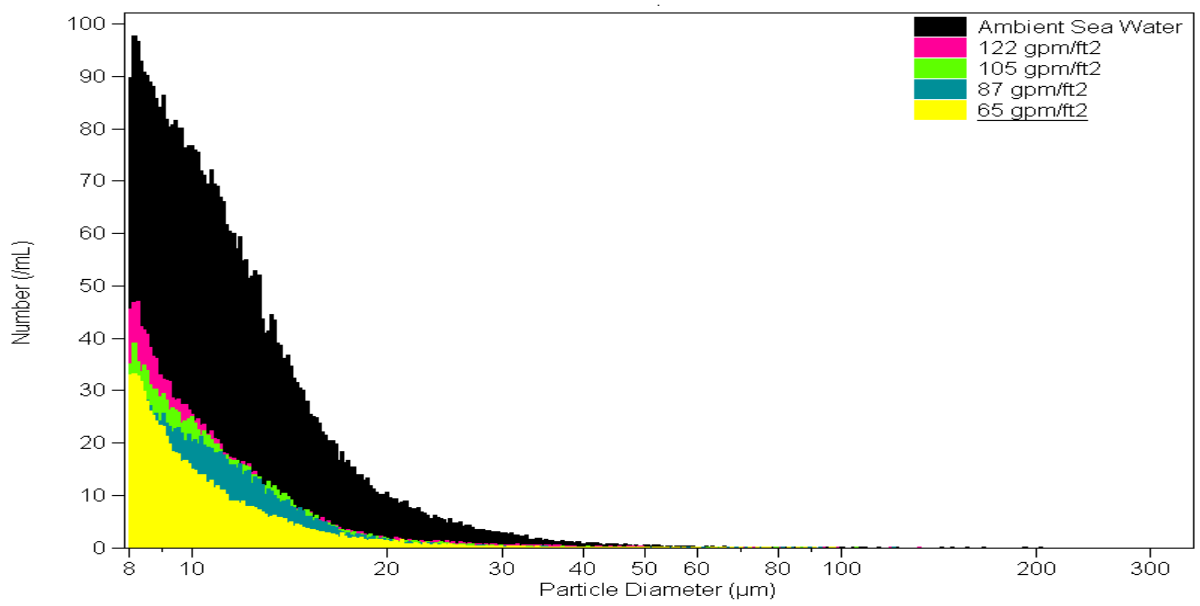


Figure 28. Particle size distributions in seawater passed through Silica Sand (0.9 to 1.0 mm) at various loading rates.

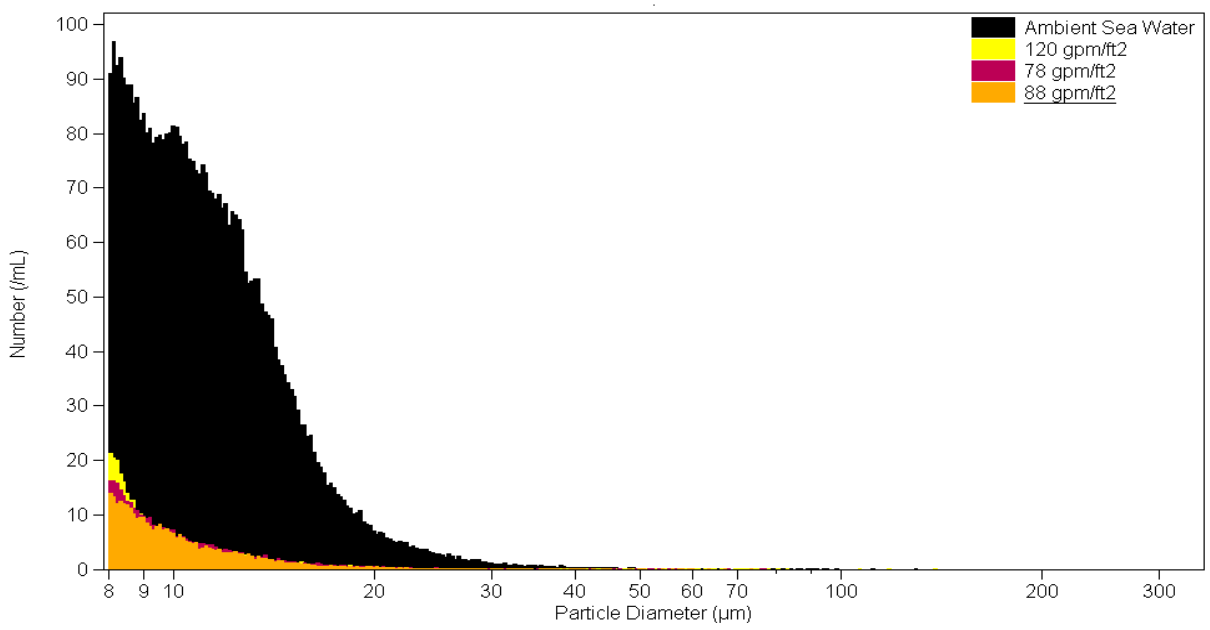


Figure 29. Particle size distributions in seawater passed through Silica Sand (0.30 to 0.45 mm) at various loading rates.

after the filtration. The outcome does not seem to be considerably affected by the type of media used. It is also evident that an increase in the loading rate results in an increase in the number of particles in the distribution. Moreover, after increasing the flow rate to a possible maximum, the size distribution of particles still appears to be lower than the ambient seawater sample, confirming removal. It appears that the largest removal takes place in particles of 10 to 30 μm particle size.

Figure 30 illustrates the percentage removal of particles (8 to 240 μm) with Silica Sand (0.45 to 0.55 mm) compared to the loading rate. As expected, the number of particles per ml increases with the loading rate, decreasing the percentage removal.

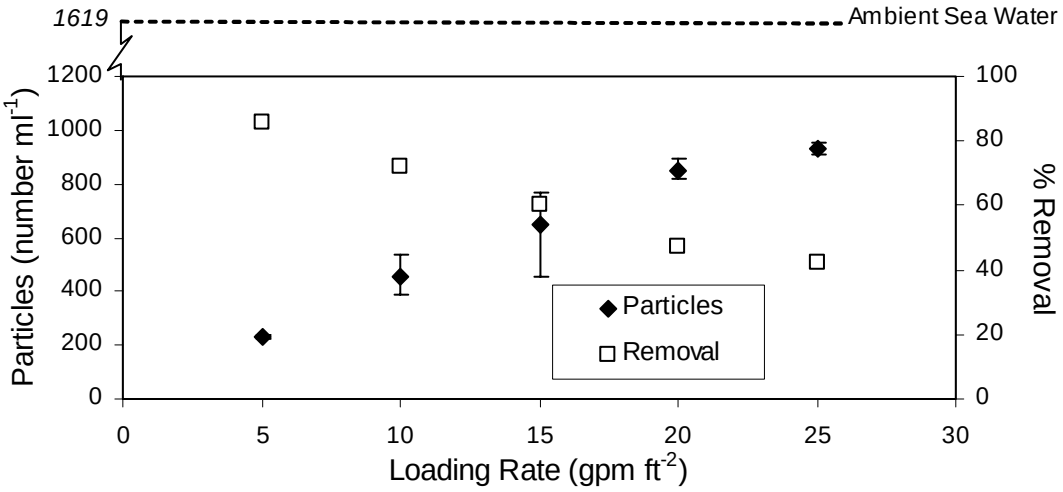


Figure 30. Removal of particles (8 to 240 μm) with Silica Sand (0.45 to 0.55 mm).

Table 17 shows the relationship between total number of particles in the ambient seawater and the loading rate distributions, calculated for particle sizes from 8 to 240 μm, for all samples. There appears to be a great difference in the number of particles per ml before and after treatment regardless of the media type, suggesting that there are fewer particles after treatment.

Finally and to complement the above results, Figures 31 and 32 show the correlation between the pressure drop and the flux for every treatment. There appears to be a direct effect on the flux as the unit pressure drop increases.

Table 17. Effect of loading rate on the total number of particles per ml.

No Media		Silica Sand (0.80 – 1.2 mm)		Silica Sand (0.45 – 0.55 mm)	
Loading rate (gpm ft^{-2})	Total number ml^{-1}	Loading rate (gpm ft^{-2})	Total number ml^{-1}	Loading rate (gpm ft^{-2})	Total number ml^{-1}
Ambient	1640	Ambient	3058	Ambient	1619
Run 1	1366	26	19421	25	931.8
Run 2	1467	22	1800	20	851.1
Run 3	1407	16	1337	15	645.6
Run 4	1363	6	879.7	10	452.6
				5	228.7
Anthracite (0.95 – 1.05 mm)		**Silica Sand (0.9 – 1.0 mm)**		**Silica Sand (0.30 – 0.45 mm)**	
Loading rate (gpm ft^{-2})	Total number ml^{-1}	Loading rate (gpm ft^{-2})	Total number ml^{-1}	Loading rate (gpm ft^{-2})	Total number ml^{-1}
27.1	1305	122	1370	120	362.5
22.5	1012	105	1246	78	361.8
18.4	800.8	87	1111	88	328.8
15.3	726.7	65	851.51		

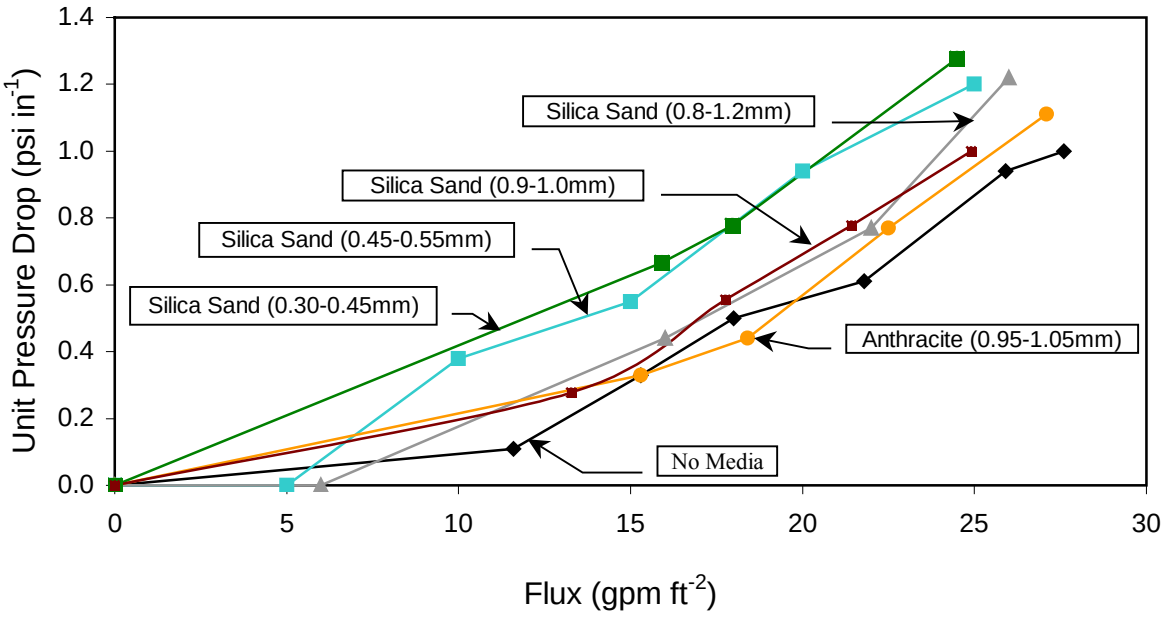


Figure 31. Pressure drop in media filter with various media (9 in deep).

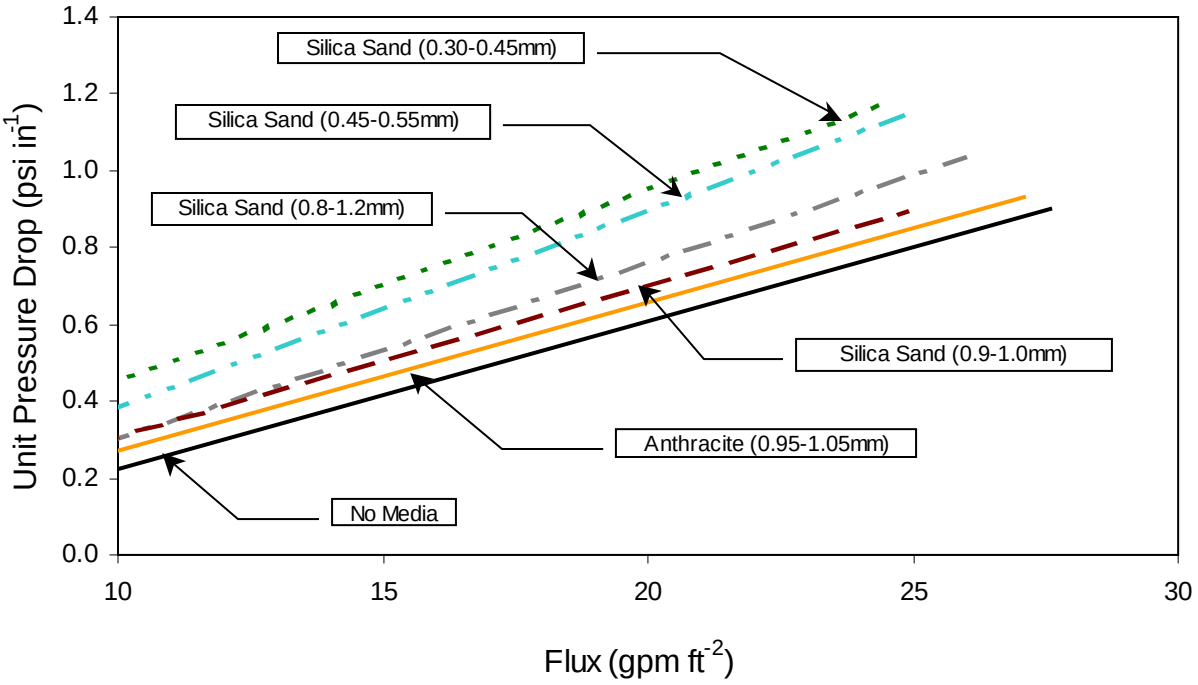


Figure 32. Trendlines of pressure drop in media filter with various media.

Conclusions

Experiments were run at large-scale to evaluate alternatives to ballast exchange at a dockside facility at the University of Miami. The facility included commercially available unit processes, specifically a hydrocyclone, a 50 µm self-cleaning screen, and UV treatment system. These unit processes were connected in such a way that the results of each unit process could be evaluated under different seawater loading conditions. A broad spectrum of biological and biochemical analysis were performed during testing to evaluate the efficiency of treatment of each unit process, singularly and in combination. The principle variable explored during the first phase of the testing protocol was the effect of increased turbidity (suspended material) on unit process efficiency. The levels of turbidity were varied from ambient seawater (approximately 5 NTU) to as high as 95 NTU by injecting clay slurries utilizing a high pressure, low volume pumping system. The main variable investigated during the second phase of the testing protocol was the effect of water color on unit process efficiency, specifically UV treatment.

Treatment efficiency of each unit process was monitored by determining the change in populations of microorganisms, including bacteria and phytoplankton, as well as macrozooplankton and larvae. In addition, concentrations of ATP and protein were monitored before and after treatment to determine if these biochemical parameters would be useful in determining treatment efficiencies.

Two physical separation processes were compared: a hydrocyclone and a 50 μm self-cleaning screen. Results of all the analyses clearly showed that the hydrocyclone was not effective at removing any of the organisms monitored except under conditions of very high turbidity loading. It was theorized that under those conditions when a large amount of clay material was added to enhance the turbidity, some coagulation of organisms occurred thus allowing for some removal through the hydrocyclone. In contrast to the hydrocyclone, the screen was very effective at removing the different macrozooplankton analyzed. Because the screen was 50 μm in size, none of the microbiota were removed by the screen during the testing process. In general, the biochemical analyses (ATP) reflected the high levels of removal of zooplankton and larvae observed with the screen system. However, more work is required to refine this type of analysis so that reliable and reproducible data can be collected.

UV treatment of seawater with augmented suspended solids resulted in variable outcomes. In all cases the UV treatment reduced bacterial populations to low levels, even in the presence of high levels of suspended solids. However, it should be noted that even at high experimental levels of turbidity, the dose delivered by the UV unit was substantial and adequate for inactivating bacterial populations common in seawater. There was also some indication that UV treatment affected the phytoplankton population; however, longer growth experiments of the phytoplankton after radiation will be required to define these effects. In a second set of experiments with enhanced water color, bacteria were not significantly reduced when treated with a relatively low UV dose as would be expected in waters of high water color. This indicates the potential for naturally occurring high levels of water color to degrade the effect of UV treatment.

Analyses were also made on treated water 18 h after treatment. In all cases it was observed that regrowth of microorganisms (bacteria) occurred during the 18 h period. It was evident that essentially all of the inactivation observed immediately after treatment with the UV system was reversed, and in fact, bacterial populations were generally higher after 18 h than before irradiation treatment. The phytoplankton appeared to decrease in viability during the 18 h incubation period indicating that some damage to these organisms occurred due to the UV treatment. In the second set of experiments with longer storage times, significant decreases in bacterial numbers and phytoplankton biomass were noted in both ambient and UV treated water samples held for 6 d in the dark. Exposure to ambient light for 24 h after the dark storage period resulted in either a decrease or no change in bacterial abundances and phytoplankton biomass.

Preliminary experiments utilizing a media filter showed that high media loading rates, with minor head loss increases, could be achieved. Concurrent particle analyses demonstrated that product water quality did not significantly degrade until extremely high flows were attained. These observations indicate that some form of media filtration could be an effective ballast water treatment process and would have advantages over screen filtration because of hydraulic and plugging considerations.

Recommendations

Based on the results of experimentation undertaken in this project, the following recommendations are made:

- Use of commercially designed hydrocyclones should not be considered as an efficient treatment mechanism for removing organisms of concern from seawater systems.

- Further environmental effects on UV treatment should be explored to more thoroughly define treatment efficacy of this unit process.

- Treatment effectiveness of other types of filter and screen systems should be explored, as it is clear that this treatment process is efficient at precluding the transport of some types of unwanted organisms.

- Operational characteristics of filtration and screen systems should be explored to determine if these units can be operated on a continuous basis on board ships.

- Treatment process efficiencies determined in these experiments should be applied to the development of a ballast water discharge standard.

- Experimental methods used in these experiments should be considered for use in ballast water treatment system certification tests that may be conducted in the future.

References

American Public Health Association, American Water Works Association, Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater,* 20th ed. American Public Health Assoc., Washington, D.C.

Arar, E.J. and G.B. Collins. 1992. In vitro Determination of Chlorophyll *a* and Phaeophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence. Method 445.0 In: *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples*. Environmental Monitoring Systems Laboratory, Office of Research and Development. U. S. Environmental Protection Agency. Cincinnati, OH.

Ascherson, N. 1996. *Black Sea*. Jonathon Cape Inc., London, England.

Ashjian, C.J., S.L. Smith and P.V.Z. Lane. 1995. The Northeast Water Polynya during summer 1992: Distribution and aspects of secondary production of copepods. J. Geophys. Res. 100: 4371-4388.

Ashjian, C., S. Smith, F. Bignami, T. Hopkins and P. Lane. 1997. Distribution of zooplankton in the Northeast Water Polynya during summer 1992. J. Mar. Sys. 10: 279-298.

Australian Quarantine and Inspection Service. 1993. Shipping Ballast Water Trials on the Bulk Carrier M.V. Iron Whyalla. Report No. 2 in Ballast Water Research Series. Canberra, Australia: Rigby, G., I. Steverson, and G. Hallegraeff for the Australian Quarantine and Inspection Service.

Australian Quarantine and Inspection Service. 1994. Ballast Water Ports and Shipping Study. Report No. 5 in Ballast Water Research Series. Canberra, Australia: Kerr, S. for the Australian Quarantine and Inspection Service.

Australian Quarantine and Inspection Service. 1995. Ballast Water Ports and Diseases Study. Report No. 7 in Ballast Water Research Series. Canberra, Australia: Herfort, A., and S. Kerr for the Australian Quarantine and Inspection Service.

Cangelosi, A.A., I.T. Knight, M. Balcer, J. Carlton, X. Gao and D. Reid. 1999. Biological test results from the Great Lakes ballast technology demonstration project. Proceedings: *First National Conference on Marine Bioinvasions*, MIT, Cambridge, MA.

Carlton, J.T., D.M. Reid and H. Van Leeuwen. 1995. The role of shipping in the introduction of nonindigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. U.S. Coast Guard Report No. CG-D-11-95. Washington, D.C.

Carlton, J.T. 1999. The global consequences of not managing ballast water. Presented at a workshop on: *Our Voyage Towards Better Ballast Water Management* IMO, MEPC 43/4 Meeting. London, England.

Cheer, S., J. Gentile and C.S. Hegre. 1974. Improved methods for ATP analysis. Analyt. Biochem. 60:102-114.

Dorsey, T.E., P.W. McDonanld and O.A. Roels. 1978. Measurements of phytoplankton-protein content with the heated biuret-Folin assay. J. Phycol.14:167-171.

Flagg, C.N. and S.L. Smith. 1989. On the use of the acoustic Doppler current profiler to measure zooplankton abundance. Deep-Sea Res. 36: 455-474.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologica 32:79-99.

Hay, C.H. and D. Tanis. 1998. Mid-Ocean Ballast Water Exchange: Procedures, Effectiveness, and Verification. Cawthron Report No. 468, Cawthron Institute, New Zealand.

Hitchcock, G.L., C. Langdon and T.J. Smayda. 1987. Short-term changes in the biology of a Gulf Stream warm-core ring: Phytoplankton biomass and productivity. Limnol. Oceanogr. 32:919-928.

Holm-Hansen, O. 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. 14:740-747.

Holm-Hansen, O. and C.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11:510-519.

Karl, D.M. and P.A. LaRock. 1975. Adenosine triphosphate measurement in soil and marine sediments. J. Fish Res. Bd. Can. 32:599-607.

Lane, P.V.Z., S.L. Smith and E.M. Schwarting. 1993. Zooplankton data report: vertical distributions of zooplankton in the Norwegian and Greenland Seas during summer, 1989. BNL-49358 Informal Report, Brookhaven National Laboratory, Upton, NY 11973. 158 pp.

Lane, P.V.Z., S.L. Smith and C.J. Ashjian. 1995. Zooplankton data report: distribution of zooplankton in the Northeast Water Polynya (NEWP) during summer, 1992. RSMAS Technical Report 95-002. Div. of Marine Biology and Fisheries, RSMAS, University of Miami, Miami, FL 33149. 139 pp.

Lane, P.V.Z., S.L. Smith and C.J. Ashjian. 1996. Zooplankton data report: distribution of zooplankton in the Northeast Water Polynya (NEWP) during summer, 1993. RSMAS Technical Report 96-007. Div. of Marine Biology and Fisheries, RSMAS, University of Miami, Miami, FL 33149. 96 pp.

Ludin, A. and A. Thore. 1975. Comparison of methods for extraction of bacterial adenosine nucleotides determined by firefly assay. Appl. Microbiol. 30: 713-731.

Maranda, Y. and G. Lacroix. 1983. Temporal variability of zooplankton biomass (ATP content and dry weight) in the St. Lawrence Estuary: Advective phenomena during neap tide. Mar. Biol. 73: 247-255.

Matheickal, J.T., T.D. Waite and M. Holmes. 1999. Systems for evaluation of ship-board ballast water treatment technologies for preventing transfer of unwanted organisms. Proceedings: *First National Conference on Marine Bioinvasions.* MIT, Cambridge, MA.

Millie, D.F., O.M. Schofield, G.J. Kirkpatrick, G. Johnsen, P.A. Tester and B.T. Vinyard. 1997. Detection of harmful algal blooms using photopigments and absorption signatures: A case study of the Florida red tide dinoflagellate, *Gymnodinium breve*. Limnol. Oceanogr. 42:1240-1251.

Mills, E.L., J.H. Leach, J.T. Carlton and C.L. Secor. 1993. Exotic species in the Great Lakes: A history of biotic crises and anthropogenic introductions. J. Great Lakes Res. 19:1-54.

Montgomery, D.C. 1997. *Design and Analysis of Experiments*, 4th ed. Wiley, New York, N.Y.

Nalepa, T.F. and D.W. Schloesser (eds). 1993. *Zebra Mussels: Biology Impacts and Control*. Lewis Publishers, Inc. (CRC Press). Boca Raton, FL.

National Research Council. 1996. *Stemming the Tide*. National Academy Press, Washington, D.C.

Neter, J., M.H. Kutner, C.J. Nachtsheim and W. Wasserman. 1996. *Applied Linear Statistical Models*, 4th ed. Richard D. Irwin, Homewood, IL.

Patterson, J., P. Brezonik and H. Putman. 1970. Measurement and significance of adenosine triphosphate in activated sludge. Environ. Sci. Technol. 4:569-575.

Postel, L., H. Fock and W. Hagen. 2000. Biomass and abundance. *In* Harris, R. P., P. H. Wiebe, J. Lenz, H. R. Skjoldal and M. Huntley, eds. *ICES Zooplankton Methodology Manual*, Academic Press. 684 pp.

Shapiro, S.S. and M.B. Wilk. 1965. *An analysis of variance test for normality (complete samples)*. Biometrika 52:591-611.

Smith, R.C., Baker, K. S. and Dustan, P. 1981. Fluorometric techniques for the measurement of oceanic chlorophyll in the support of remote sensing. SIO Ref. 81-17, *Scripps Institute of Oceanography*, La Jolla, CA, 14 pp.

Smith, S.L., P.V.Z. Lane and E.M. Schwarting. 1985. Zooplankton data report: shelf-edge exchange processes (SEEP) I. Technical Report Number BNL 36260, Brookhaven National Laboratory, Upton, NY 11973, 74 pp.

Smith, S.L. and P.V.Z. Lane. 1988. Grazing of the spring diatom bloom in the New York Bight by the calanoid copepods *Calanus finmarchicus, Metridia lucens* and *Centropages typicus*. Cont. Shelf. Res. 8: 485-509.

Appendices

Appendices A through E are available upon request and are organized as follows:

Appendix A – Turbidity Experiments Test Data
Appendix B – Turbidity Experiments Statistical Analyses
Appendix C – Color Experiments Test Data
Appendix D – Color Experiments Statistical Analyses
Appendix E – Particle Size Analyses